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Non-random inheritance of organellar genomes in symmetric and asymmetric somatic hybrids between *Lycopersicon esculentum* and *L. pennellii*

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Summary. The organization of the mitochondrial genome and the genotype of the chloroplast genome was characterized using restriction fragment length polymorphisms in a population (82 individuals) of symmetric and asymmetric somatic hybrids of tomato. The protoplast fusion products were regenerated following the fusion of leaf mesophyll protoplasts of *Lycopersicon esculentum* (tomato cv 'UC82') with suspension cell protoplasts of *L. pennellii* that had been irradiated with 5, 10, 15, 25, 50, or 100 kRads from a gamma source. The chloroplast genome in the somatic hybrids showed a random pattern of inheritance, i.e., either parental genome was present in equal numbers of regenerants, while in asymmetric somatic hybrids, the chloroplast genotype reflected the predominant nuclear genotype, i.e., tomato. The mitochondrial genome in the symmetric somatic hybrids showed a non-random pattern of inheritance, i.e., predominantly from the *L. pennellii* parent; asymmetric somatic hybrids had more tomato-specific mitochondrial sequences than symmetric somatic hybrids. The non-random inheritance of the chloroplast and mitochondrial DNA in these tomato protoplast fusion products appears to be influenced by the nuclear background of the regenerant.

Key words: Tomato – Nucleo-cytoplasmic – Gamma radiation – Mitochondrial DNA – Chloroplast DNA

Introduction

Protoplast fusion can allow the independent inheritance of chloroplast and mitochondrial genomes (review Galun and Aviv 1983). In somatic hybrids and cybrids, usually only one of the parental chloroplast genomes is inherited, the result of sorting out of the parental organelles during cell divisions following protoplast fusion (Sidorov et al. 1981; Menczel et al. 1982). Hybrid chloroplast genomes have only been found in regenerants following strong selection pressure (Medgyesy et al. 1985; Thanh and Medgyesy 1989). The mitochondrial DNA (mtDNA) in fusion products frequently has a novel organization arising from recombination between the parental genomes (review Galun and Aviv 1983).

Asymmetric somatic hybrids have been constructed in Solanaceous species by fusing recipient protoplasts with irradiated donor protoplasts (Imamura et al. 1987; Gleba et al. 1988; Famelaer et al. 1989; Melzer and O'Connell 1990; 1992; Piastuch and Bates 1990; Wijbrandi et al. 1990). Depending on the radiation dose, the nuclear genetic information is fragmented or effectively inactivated. Irradiation of the donor protoplasts with high doses virtually eliminates the donor nuclear genome in fusion products, named cytoplasmic hybrids or cybrids. Cybrid constructions have also been made in Solanaceous species (Sidorov et al. 1981; Menczel et al. 1982; Fluhr et al. 1983; Glimelius et al. 1986; Aviv and Galun 1988; Bonnema et al. 1991; Perl et al. 1990). The use of radiation in fusion constructions to transfer cytoplasmic genomes presumes that a sufficient number of organellar genomes remain intact in the irradiated cell.

The assumption has been that in the absence of selection, organellar genomes are randomly inherited and unaffected by experimental treatments of the parental protoplasts i.e., ionizing radiation or iodoacetate (review Medgyesy 1990). In cases where the regeneration of hybrid plants following protoplast fusion uses a chloroplast-encoded resistance character (Menczel et al. 1982; Fluhr et al. 1983; among others); a non-random inheri-

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tance of the cpDNA in the fusion products is expected. However, there are cases where the inheritance of the organellar genome appears to be non-random and where no selection pressure has been deliberately applied (Thanh et al. 1988; Levi et al. 1988; San et al. 1990). Explanations for non-random inheritance have included the tissue source of the protoplasts used in the fusion, differential replication rates for the organelles, or intergeneric nucleo-cytoplasmic incompatibility. The data to support the donor source explanation is conflicting, and there have been no studies to directly test differential replication rates as a mechanism for non-random organelle inheritance (review Rose et al. 1990). Direct support for a genetic basis for non-random organelle inheritance has been obtained in the extreme cases where certain combinations of genomes are not recovered or produce non-viable phenotypes (Thanh et al. 1988; Thanh and Medgyesy 1989; Kushnir et al. 1991).

We decided to test whether the inheritance of chloroplast and mitochondrial genomes was influenced by the nuclear background of the protoplast fusion product. We have generated a collection of tomato symmetric, asymmetric and cybrid protoplast fusion products between tomato (*Lycopersicon esculentum*) and *L. pennellii*. We define symmetric somatic hybrids as regenerants that contain essentially equal amounts of nuclear genetic information from the two protoplast fusion partners, and asymmetric somatic hybrids as regenerants that contain unequal amounts of nuclear genetic information from the two protoplast fusion partners. Cybrids in this context are special cases of asymmetric somatic hybrids since they contain the nuclear genome of only one of the protoplast fusion partners. In earlier studies we compared the organization of the organellar genomes in *L. esculentum* + *L.pennellii* symmetric somatic hybrids and cybrids (Bonnema et al. 1991; Wachocki et al. 1991) and observed more tomato-specific mtDNA sequences in cybrids than in somatic hybrids. We recently regenerated a large number of asymmetric somatic hybrids following the fusion of tomato leaf protoplasts with *L. pennelli* suspension cell protoplasts that had been irradiated with a range of doses from a gamma source. The nuclear composition of the regenerants was determined using restriction fragment length polymorphism (RFLP) markers (Melzer and O'Connell 1992). In this paper the composition of the mitochondrial and chloroplast genomes in these same regenerants is presented. A comparison of the nuclear genotype and the organellar genotypes was analyzed statistically to determine if there was a possible genetic basis for the non-random inheritance of both organellar genomes.

Table 1. Description of the mitochondrial probes. The diagnostic restriction enzymes which identify species-specific RFLPs, tomato (E) or *L. pennellii* (P), the total species-specific sequences in kb, as well as the genes encoded on the clone are listed

Clone	Digest	Species-specific RFLPs (kb)		Mitochondrial genes
		E	P	
Cosmid A1	*Hin*dIII	6.8	8.8	*rrn26, atp9*
Cosmid A2	*Sma*I	1.8	2.0	*rrn18, rrn5, atpA, atp6*
Cosmid A2	*Bam*HI	1.4	1.6	
Cosmid A3	*Hin*dIII	7.3	16.5	3.2-kb "repeat"
Cosmid B3	*Hin*dIII	22.5	13.8	*rrn18, rrn5*
Cosmid C3	*Sal*I	7.5	4.3	*rrn26*
Cosmid D9	*Hin*dIII	3.9	10.8	*atp9*
2D4	*Sal*I	0.0	6.8	

Materials and methods

Plant material

Seeds of *Lycopersicon pennellii* LA 716, collected in Atico, Peru, were generously provided by C. Rick, Tomato Genetics, Stock Center, University of California, Davis. Seeds of *L. esculentum* cv 'UC82B' were from Petoseed Co. The construction of the protoplast fusion products analyzed in this report have already been described (Bonnema et al. 1991; Melzer and O'Connell 1992). Briefly, protoplasts were isolated from leaf mesophyll tissue of 'UC82', treated with iodoacetamide, and fused using polyethylene glycol to protoplasts isolated from suspension-cultured cells of *L. pennellii*. The protoplasts from *L. pennellii* were exposed to varying doses (5–100 kRads) of ^{60}Co before fusion. The protoplasts were cultured and regenerated as described earlier (O'Connell and Hanson 1987; Bonnema et al. 1991).

DNA isolation and description of nuclear probes

Total DNA was extracted from fresh leaf tissue as described by Doyle and Doyle (1989), with the addition of a CsCl centrifugation after the isopropanol precipitation. DNA isolation, restriction, Southern transfer, and hybridization conditions for the nuclear probes have been described previously (Melzer and O'Connell 1992). The 35 molecular loci used in this study were: chromosome 1, CD15, CD24, CD28; chromosome 2, CD35, CD37, *Rbcs-1, Cab-1*; chromosome 3, *Rbcs-2*, CD6, CD13, CD31; chromosome 4, CD39, TG15, *Pgm-2*; chromosome 5, CD31, CD31, TG69; chromosome 6, TG54, TG115, CD13, CD14; chromosome 7, TG13A; chromosome 8, CD40; chromosome 9, CD8, CD32B, TG18; chromosome 10, CD32A, CD38A, TG63; chromosome 11, TG30, TG46; chromosome 12, CD2, CD6, CD19, CD27, *Pgi*-1 (Bernatzky and Tanksley 1986; Mutschler et al. 1987; Zamir and Tanksley 1988). The isozyme activities were detected as described earlier (O'Connell and Hanson 1987). Individual regenerants were scored at between 20 and 24 of these loci.

Description of organellar probes

The methods described by Bonnema et al. (1991) were used for Southern hybridization using organellar probes. A 27-kb *Sal*I fragment of the tomato chloroplast (cp) genome was cloned into pUC8 and used to probe *Hin*dIII digests of total DNA. This 27-kb *Sal*I fragment contains the inverted repeat of the cpDNA (Phillips 1985) and identifies a species-specific RFLP. Seven non-overlapping cosmid clones containing tomato mtDNA (Wachocki et al. 1991) and clone 2D4, a 2.1-kb *L. pennellii Sal*I

Table 2. The nuclear composition and organellar genotype of tomato asymmetric and symmetric somatic hybrids. The regenerants are grouped by the dose of radiation received by *L. pennellii* prior to fusion. The nuclear genotype (nucDNA) indicates the number of loci scored as heterozygous (H) or homozygous tomato (E); the chloroplast genotype is based on the scoring of a single species-specific RFLP. The mtDNA is described as percent tomato (E), *L. pennellii* (P), or novel fragment (R), based on a weighted percent of species-specific or novel fragments detected by the mtDNA specific probes

Name	NucDNA		MtDNA			CpDNA
	H	E	%E	%P	%R	
UC82(+) *L. pennellii*-5 kRads						
4	21	0	0	100	0	E
5A	21	0	0	84	16	P
11A	21	0	0	100	0	E
13B	21	0	0	100	0	P
57	21	0	17	69	14	E
126	21	0	0	83	17	E
203	21	0	17	68	15	E
207B	21	0	0	84	16	P
208	21	0	0	100	0	P
225B	21	0	0	100	0	P
265B	21	0	28	40	32	P
602H	21	0	0	84	16	E
630A	21	0	0	84	16	E
57B	21	0	0	84	16	E
13A	21	0	0	100	0	P
UC82(+) *L. pennellii*-10-kRads						
36	20	0	0	100	0	E
61B	20	0	0	100	0	E
94	20	0	0	100	0	E
102A	20	0	0	84	16	E
223A	20	0	6	94	0	E
242	20	0	0	84	16	E
261	20	0	0	85	15	P
265A	20	0	0	84	16	P
289	20	0	0	84	16	E
291B	20	0	0	100	0	P
330B	20	0	0	100	0	E
467B	20	0	0	100	0	E
473B	20	0	18	75	7	E
509	20	0	0	100	0	E
628	20	0	0	84	16	P
644B	20	0	0	84	16	P
UC82(+) *L. pennellii*-15 kRads						
16	21	0	0	100	0	P
53B	21	0	0	100	0	P
60C	20	1	0	84	16	E
74A	21	0	0	100	0	P
79C	21	0	0	100	0	E
80	21	0	0	100	0	P
84	20	1	0	100	0	P
89B	21	0	0	100	0	P
97F	21	0	0	84	16	P
165C	20	0	0	82	18	E
189B	20	0	0	84	16	E
255B	19	1	5	80	15	E
256A	20	0	0	84	16	E
263A	6	14	0	84	16	P
301B	20	0	0	84	16	P
303A	20	0	0	84	16	E
102B	9	12	43	29	28	E
UC82(+) *L. pennellii*-25 kRads						
7C	20	2	0	82	18	E
9B	21	1	0	100	0	P
13	22	1	47	44	9	E
14B	23	0	0	100	0	P
19D	23	0	0	100	0	E
20A	23	0	0	100	0	P
24B	23	0	0	84	16	E
43	22	1	0	100	0	P
56	23	0	0	100	0	E
63B	23	0	0	85	15	E
108	22	1	35	26	39	E
118C	23	0	0	100	0	E
201A	22	1	0	84	16	E
217	23	0	0	84	16	E
226A	23	0	0	84	16	P
200D	1	20	100	0	0	E
1	2	19	100	0	0	E
UC82(+) *L. pennellii*-50 kRads						
1	0	21	0	84	16	E
2	0	20	100	0	0	E
3	1	20	100	0	0	E
10	1	15	3	80	17	E
UC82(+) *L. pennellii*-100 kRads						
1	17	4	12	74	14	E
76	3	18	4	65	31	E
81	1	16	0	85	15	E
92A	1	22	75	0	25	E
92B	1	12	65	0	35	E
100A	0	23	15	72	13	E
100B	0	17	10	72	13	E
100C	0	17	11	76	13	E
100D	0	15	15	72	13	E
100E	0	16	15	72	13	E
121	1	19	0	86	14	E
122	0	12	0	85	15	E
16A	0	16	87	0	13	E

fragment that occurs in multiple copies in the genome (McClean and Hanson 1986), were used to analyze the mtDNA of the regenerants. Table 1 lists the tomato mtDNA cosmid clones, the insert size, and the size of the species-specific fragments identified in the indicated restriction digestions.

Statistical analysis

Third-order polynomial regression models were fit for percentage tomato nuclear DNA (%E nucDNA) and percentage tomato mtDNA (%E mtDNA) using radiation dose as the predictor variable, and the correlation between %E nucDNA was calculated. In addition, two logistic regression models (Neter et al.

1989) were fit for cpDNA with dose as the predictor variable in one model and %E nucDNA in the other. Logistic regression, a non-linear regression procedure, uses weighted least squares to model the natural log of the ratio of percent individuals with tomato cpDNA to percent with *L. pennellii* cpDNA. All analyses were done with the SAS software package (SAS Institute 1985).

Results

Nuclear genotype of the fusion products

The nuclear genotype of the regenerated fusion products was analyzed using isozyme activities for phosphoglucomutase and phosphoglucoisomerase and RFLP analyses using tomato cDNA or genomic clones as probes (Melzer and O'Connell 1992). The number of loci that scored as homozygous tomato (E) or heterozygous (H) for each regenerant is presented in Table 2. None of the individuals scored as homozygous *L. pennellii* at any of the loci. From this list it is clear, that as the radiation dose used to treat the *L. pennellii* protoplast fusion partner increased, the degree and frequency of asymmetry in the regenerated fusion products also increased. All the fusion products recovered following fusion of 5- or 10-kRad-treated *L. pennellii* protoplasts were symmetric somatic hybrids. At higher doses, 15, 25, 50 and 100 kRads, the percentage of protoplast fusion products that were asymmetric increased: 29%, 47%, 100%, and 100%, respectively. We included cybrids in the category of asymmetric fusion products. Descriptions of the cybrids and non-cybrid asymmetric fusion products have been presented earlier (Bonnema et al. 1991; Melzer and O'Connell 1992).

Chloroplast genotype

The chloroplast genotype of the regenerants was determined using a cloned 27-kb *Sal*I fragment of tomato cpDNA (Phillips 1985). This 27-kb *Sal*I fragment hybridizes to several *Hin*dIII fragments, three of which display useful polymorphisms; 10- and 3.8 kb fragments in tomato cpDNA and a 14-kb fragment in *L. pennellii* cpDNA. A typical Southern analysis using this probe is shown for 6 regenerants of the fusion *L. pennellii* 15-kRad (+) tomato in Fig. 1. Four of the 6 individuals in Fig. 1 inherited the cpDNA of the tomato parent. A summary of the chloroplast genotypes of all of the regenerants is presented in Table 2.

The cpDNA in the regenerants recovered following fusion of 5-, 10-, 15-, or 25-kRad-treated *L. pennellii* protoplasts showed essentially random inheritance of either parental chloroplast genome: 53%, 69%, 47%, and 70%, respectively, inherited the tomato cpDNA. However, all of the regenerants recovered following the fusion of 50- or 100-kRad-treated *L. pennellii* protoplasts inherited the tomato cpDNA.

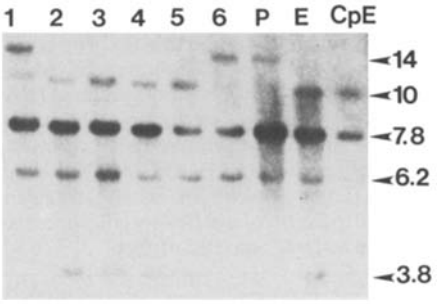

Fig. 1. Identification of the chloroplast genomes present in regenerants of the fusion of *L. pennellii* 15 kRad (+) tomato. All lanes contain 8 µg of total DNA restricted with *Hin*dIII. The Southern blot was probed with a cloned 27-kb *Sal*I fragment to tomato cpDNA. *Lanes E* and *P* contain DNA from *L. esculentum* cv 'UC82' and *L. pennellii*, respectively. The *lanes* containing DNA from the regenerants are labeled 1–6; the sizes of the hybridizing fragments are indicated in kb

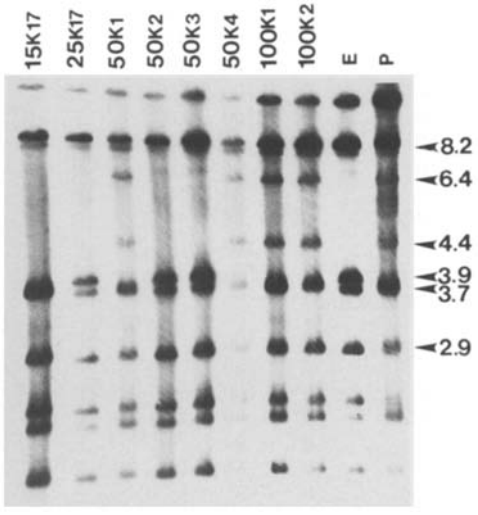

Fig. 2. Identification of the mitochondrial sequences which hybridize to the cosmid clone D9 present in 8 regenerants of fusions of tomato (+) *L. pennellii* irradiated with 15, 25, 50 or 100 kRads. All lanes contain 8 µg of total DNA restricted with *Hin*dIII. *Lanes E* and *P* contain DNA from *L. esculentum* cv 'UC82' or *L. pennellii*. The *lanes* containing DNA from the regenerants are labeled with the *radiation dose* given to *L. pennellii* followed by an *identifying number*. The sizes of the hybridizing fragments are indicated in kb

The mitochondrial genome

A collection of cloned tomato mtDNA fragments was used for the analysis of the mitochondrial genome of the regenerants (Table 1). Altogether, these clones account for at least 230 kb of tomato mtDNA and are predicted to cover at least 60% of the mitochondrial genome, assuming the genome is between 300 and 400 kb (Hause et al. 1986; McClean and Hanson 1986).

The hybridization pattern of cosmid D9 to *Hin*dIII-digested DNA of several regenerants and the parents is shown in Fig. 2. This probe hybridized to a tomato-

specific *Hin*dIII fragment of 3.9 kb and to two *L. pennellii*-specific *Hin*dIII fragments of 6.4 ad 4.4 kb. The *L. pennellii*-specific fragments were present in a lower stoichiometry than the other fragments that hybridized to this probe. The mtDNA organization of the regenerants 25k17, 50k2, and 50k3 was identical to tomato, while the mtDNA organization of the regenerants 50k1, 50k4, 100k1, and 100k2 was identical to *L. pennellii*. *Hin*dIII-digested DNA of regenerant 15k17 lacked all parental-specific *Hin*dIII fragments when probed with cosmid D9. No novel or non-parental bands were observed in this particular case. The data from this Southern analysis were recorded as follows: individuals 25k17, 50k2, and 50k3 had 3.9-kb tomato-specific mtDNA, while individuals 50k1, 50k4, 100k1, and 100k2 had 10.8-kb (6.4+4.4) *L. pennellii*-specific mtDNA; and 15k17 had no species-specific mtDNA.

Equivalent Southern analyses were performed with all eight of the mitochondrial probes using one or two different restricted DNA samples. The sum of all of the tomato-specific, *L. pennellii*-specific, and novel fragments in kb was calculated. The percentage of those sequences that were tomato, *L. pennellii*, or novel was then determined. Altogether, the probes, listed in Table 1, identified 51.2 kb of tomato-specific mtDNA RFLPs and 64.6 kb of *L. pennellii*-specific mtDNA RFLPs. Therefore, if an individual were to have a 1:1 ratio of each species mtDNA, then the mtDNA would be scored as 56% P and 44% E in Table 2, since there was a slight difference in the sizes of species-specific fragments. Table 2 lists these percentages of species-specific or novel fragments for each protoplast fusion product.

With few exceptions, most of the species-specific RFLPs in the mtDNA of the regenerants were *L. pennellii* specific. For regenerants recovered following fusion of 5-, 10-, 15-, or 25-kRad-treated *L. pennellii* protoplasts, 40% had only *L. pennellii* mtDNA RFLPs. Altogether, this group of regenerants had an average of 85% *L. pennellii*-specific mtDNA sequences. None of the regenerants from the 50- or 100-kRad population had only *L. pennellii* mtDNA and this group of regenerants had on average 54% *L. pennellii*-specific mtDNA sequences.

Effect of nuclear genotype on inheritance of cpDNA and mtDNA

A cursory inspection of the results presented in Table 2 suggests that chloroplast genotype was randomly inherited from either fusion parent in the somatic hybrid regenerants and that there was a preference of the inheritance of the tomato genotype of chloroplast in the asymmetric somatic hybrids.

Logistic regression analysis showed significant relationships when either dose of radiation (Fig. 3A) or nuclear genotype (Fig. 3b) were used to predict the chloroplast genotype. The best fit was when dose was used [χ^2 (1) = 5.58, $P = 0.01$]; nuclear genotype was less significant as a predictor variable [χ^2 (1) = 2.96, $P = 0.08$]. In both cases, increase in the level of predictor (either dose or %E nucDNA) was related to an increase in the proportion of individuals with E cpDNA. Nuclear genotype is directly related to the dose of radiation used before fusion (Melzer and O'Connell 1992; Fig. 4A). Therefore, it was not clear whether the non-random inheritance of cpDNA in the asymmetric somatic hybrids was a direct result of radiation or an indirect effect of the radiation dose on nuclear genotype.

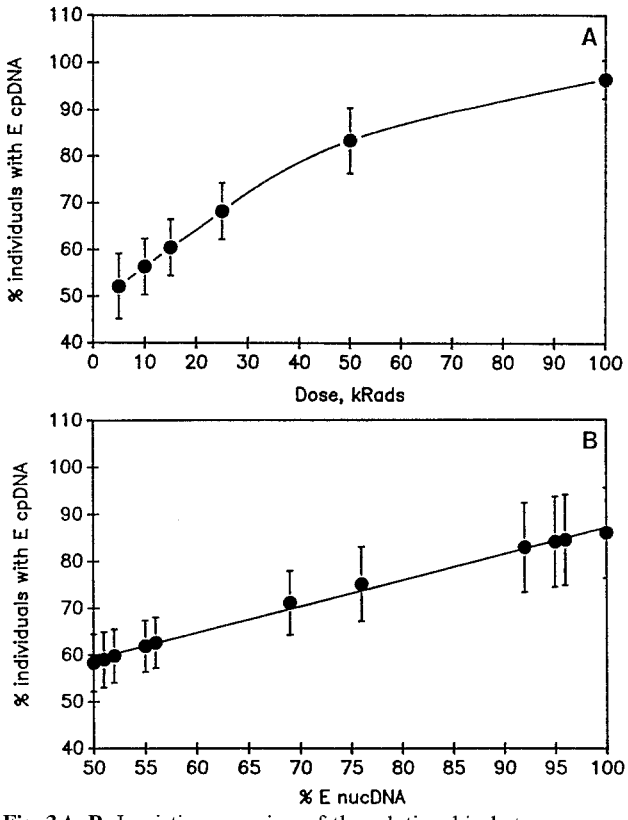

Fig. 3A, B. Logistic regression of the relationship between predicted percentage of individuals with tomato cpDNA (*E cpDNA*) and either **A** the dose of radiation received by the *L. pennellii* protoplast fusion partner or **B** the percentage of nuclear loci that scored as tomato (*%E nucDNA*)

An inspection of the organization of the mtDNA in the symmetric and asymmetric hybrids also suggested a relationship between nuclear genotype and mtDNA inheritance. As mtDNA recombination is fairly common between protoplast fusion partners, the mtDNA in the regenerants are not clearly one parental form or another, but rather unequal mixtures of parts of the two genomes. The predominant species-specific RFLPs in the mtDNA in the symmetric and asymmetric somatic hybrids was usually *L. pennellii* (Table 2). As the dose of radiation used to treat the *L. pennellii* protoplast fusion partner

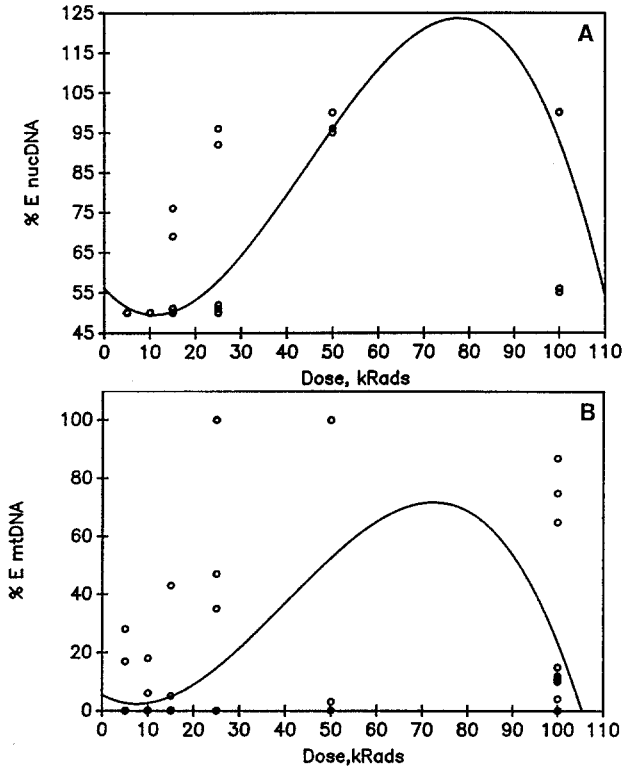

Fig. 4A, B. Third-order polynomial regression of the relationship between dose of radiation received by the *L. pennelli* protoplast fusion partner and either **A** the percentage of nuclear loci that scored as tomato (*%E nucDNA*) or **B** the percentage of mitochondrial sequences that were tomato specific (*%E mtDNA*)

increased, the percentage of *L. pennellii*-specific mtDNA sequences in the fusion product decreased. Third-order polynomial regression models were found to describe the relationship between dose of radiation and nuclear genotype and between dose of radiation and mtDNA. Plots of observed combinations of radiation dose and %E of either nucDNA or mtDNA are given in Fig. 4A and B, together with fitted regression models. The models accounted for 75% of the variability in the relationship between dose of radiation and asymmetry in nuclear genotype, but only for 22% of the variability in the relationship between dose of radiation and mtDNA inheritance. There was a significant linear association between the nuclear genotype of the protoplast fusion product and the tendency to inherit specific organizations of mtDNA, (correlation coefficient 0.5, $P = 0.0001$).

Discussion

The organellar composition of fusion products derived from the fusion of tomato (+) *L. pennellii*, irradiated with a range of doses (5, 10, 15, 25, 50, and 100 kRad ^{60}Co gamma radiation), and with known nuclear genotype, was determined. There was a direct relationship between the dose of radiation received by the *L. pennellii* protoplast and the amount of nuclear genetic information inherited in the protoplast fusion product (Fig. 4A). The inheritance of cpDNA was observed to be random in symmetric somatic hybrids and non-random in asymmetric somatic hybrids (Fig. 3B). The inheritance of mtDNA was observed to be non-random in symmetric somatic hybrids and to approach random inheritance in asymmetric somatic hybrids (Fig. 4B). In earlier studies, we proposed that a nuclear-cytoplasmic interaction might contribute to non-random organellar inheritance in tomato protoplast fusion products (Wachocki et al. 1991). Based on the results presented here, we propose that the nuclear background exerts an influence on organellar inheritance. An increase in the percentage of tomato alleles in the nucleus was accompanied by an increased likelihood of an individual inheriting the tomato chloroplast genome. Similarly, as the percentage of tomato alleles in the nucleus increased, there was an increased likelihood of an individual having more tomato-specific mtDNA sequences. There is also the possibility that these alterations in the inheritance of organellar sequences was a direct result of the radiation treatment received by the *L. pennellii* fusion parent, although this is not expected (Medgyesy 1990). We were unable to distinguish between nuclear effects and radiation-induced effects on the inheritance of the organellar DNA in this analysis. Nuclear genotype was directly affected by the radiation treatment, as expected.

In the specific combination of tomato and *L. pennellii*, the *L. pennellii* mtDNA appears to predominate in a hybrid nuclear background, while the chloroplasts segregated randomly. Similar results were obtained by San et al. (1990). In tomato (+) *L. peruvianum* somatic hybrids, the chloroplast genomes segregated randomly, while the *L. peruvianum* mtDNA was predominant. Derks et al. (1991) analyzed the cpDNA and a limited amount of mtDNA in symmetric and asymmetric somatic hybrids between tomato and *L. peruvianum*. They concluded that the inheritance of cpDNA was random and that the inheritance of mtDNA was skewed towards the *L. peruvianum* parent in the symmetric hybrids. They also suggest that irradiation affected the inheritance of the mtDNA. Levi et al. (1988) observed non-random inheritance of both mtDNA and cpDNA in somatic hybrids between tomato and *Solanum lycopersicoides*. In this case, the cpDNA in the hybrids was preferentially from the tomato parent, and the mtDNA was exclusively from the *S. lycopersicoides* parent. In all four cases of tomato protoplast fusion products with *L. pennellii*, *L. peruvianum*, and *S. lycopersicoides*, the mtDNA from the wild species was predominant over the tomato mtDNA.

We did recover regenerants that had exclusively *L.* pennellii mtDNA. The mitochondria in these fusion products may have sorted without undergoing mitochon-

drial fusion. However, in most cases, evidence of mitochondrial recombination suggests that after the cellular fusion event mitochondrial fusion and intergenomic recombination occurred, and a mitochondrial genome enriched in *L. pennellii* sequences was the most stable or preferred genome in those cells that underwent organogenesis. We suggest that more likely explanations for the dominance of *L. pennellii* mtDNA in our somatic hybrids is (1) a higher replication rate of *L. pennellii* mtDNA than tomato mtDNA or (2) the *L. pennellii* nuclear alleles concerning the nucleus-mitochondrion interaction are dominant, thus directing mtDNA segregation in the somatic hybrids.

Non-random organellar inheritance is most frequently observed in combinations of species that are sexually incompatible (Bonnett and Glimelius 1983; Levi et al. 1988; Landgren and Glimelius 1990; Perl et al. 1990). This observation suggests that the inheritance of organellar genomes in protoplast fusion products is controlled, to some extent, genetically. Potential sources of control could be nuclear-organellar incompatibility, inherent differences in organellar genome replication rates, or alternatively, the tissue source of the protoplasts (Rose et al. 1990).

Leaf mesophyll cells are relatively enriched in chloroplasts relative to suspension-cultured cells (Steele-Scott et al. 1984; Thomas and Rose 1983). One would predict that the somatic hybrids should preferentially inherit the tomato cpDNA because tomato leaf mesophyll cells were used in the fusion. We did not observe such an inheritance pattern, but rather a random pattern of inheritance of cpDNA in the symmetric somatic hybrids. Large changes in mtDNA per cell during development have not been observed (Lammpa and Bendich 1984), although tissue-cultured cells have been demonstrated to have altered organizations of mtDNA (McNay et al. 1984; Brears et al. 1989; Shirzadegan et al. 1989). In the case of mtDNA, the dominance of the *L. pennellii* mtDNA in our fusion products could be the use of *L. pennellii* suspension cells. These cells grow heterotrophically, and this may result in a more competitive form of a mitochondria.

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