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Functional cybrid plants of *Lycopersicon peruvianum* var '*dentatum*' with chloroplasts of *Lycopersicon esculentum*

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Abstract Fertile cybrid plants of three subclones, B1A, B3A, B4A were regenerated from the single colony obtained after the fusion of mesophyll protoplasts of plastome chlorophyll-deficient mutant *Lycopersicon peruvianum* var '*dentatum*' (line 3767) and γ-irradiated mesophyll protoplasts of *L. esculentum* (cv 'Quedlinburger Frühe Liebe'). Cytogenetic, isozyme, RAPD, morphological and restriction analyses all showed that the subclones had the nuclear genome of *L. peruvianum* var '*dentatum*' and plastome genome of *L. esculentum*, while the mitochondrial genome was altered. No phenotypical traits that could be taken as evidence of plastome-genome incompatibility in the cybrid subclones were observed. Genetic functionality of all subclones was proven by the backcrossing analysis. To study the functionality of the cybrid plants we also carried out an analysis of their photosynthetic system. Data on chlorophyll-a and -b content, analyses of the fluorescence induction curves, intensity of CO_2 assimilation, pigment-protein complexes and polypeptides of thylakoid membranes showed the absence of structural and functional abnormalities in the photosynthetic apparatus of the cybrid plants. We concluded that the plastome of *L. esculentum* is able to effectively interract with the nuclear genome of *L. peruvianum* var '*dentatum*' and together with the recombined chondriome can support genetic functionality of cybrid plants of the peruvianum tomato.

Key words *Lycopersicon* · Protoplast fusion · Cybrids · Nuclear-cytoplasmic interrelations

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Introduction

Genetic information in plant cells is located in three isolated genetic compartments of the nucleus, plastids and mitochondria. Plants can function as a whole organism only as a result of the close interaction between these separate genomes. Moreover, this interaction should be harmonious and balanced since it is the result of a long conjugated evolution of these cell compartments (Herrmann 1989).

The arbitrary combination of nuclear and cytoplasmic genomes of different species by sexual or somatic hybridization results as a rule in serious abnormalities in growth and the development of hybrid plants (alloplasmatic and cytoplasmic hybrids, accordingly). Unlike sexual hybridization, somatic hybridization by the fusion of isolated protoplasts enables cytoplasmic hybrids to be obtained not only between close species but also between phylogenetically remote taxons (Medgyesy 1990). In some cases new phenotypic traits uncharacteristic of the parental species have been described for such cybrids (Kofer et al. 1991; Kushnir et al. 1991; Perl et al. 1991; Ratushnyak et al. 1995; Zubko et al. 1996). The authors explain these traits as a result of incompatibility between nuclear and organelle genomes. Although the phenomenon of nuclear-cytoplasmic incompatibility was described some time ago in classic studies on *Epilobium* and *Oenothera* (Michaelis 1965; Kirk and Tilney-Basset 1978), its molecular mechanisms remain unclear. Moreover, most of the nuclear-cytoplasmic incompatibility described based on easily

observable phenotypical changes (chlorophyll deficiency, dwarfish and abnormal floral morphogenesis). The absence of visual deviations in the pigmentation, growth and development of cybrid plants whose cells contain nuclear and cytoplasmic determinants of different species, genuses and tribes and even the reproductive capacities of these plants by themselves does not indicate the absence of abnormalities of any viable physiological processes (photosynthesis and respiration in particular). Their realization necessitates a coordinated interaction of at least two different genomes (the nuclear genome and the plastome, the nuclear genome and the chondriome, respectively).

For the genus *Lycopersicon*, fertile somatic hybrids have been obtained between the cultivated tomato *Lycopersicon esculentum* and wild species that could not be crossed with the cultivated tomato via the normal sexual process (Wijbrandi et al. 1990b; San et al. 1990; Sakata et al. 1991; Bonnema and O'Connell 1992). Additionally, asymmetric somatic hybridization was performed. Sterile as well as fertile asymmetric somatic hybrids containing nuclear and cytoplasmic determinants of *L. peruvianum, L. hirsutum* and *L. pennellii* were developed (Wijbrandi et al. 1990a; Derks et al. 1992; Melzer and O'Connell 1992; Ratushnyak et al. 1993). Since the tomato is characterized by the maternal type of inheritance of cytoplasmic organelles under their sexual hybridization, an increase in genetic variability induced by the transfer of plasmogens from wild species into the cultivated tomato would be useful. In some cases, however, elimination of the nuclear material of the donor species was accompanied by abnormal nuclear-organelle interactions in the cybrids. Fertile and morphologically perfect cybrids of cultivated tomato carrying the plastome of *L. hirsutum* were obtained by Derks et al. (1992). Fertile cybrids were also obtained for the combination *Lycopersicon esculentum* + *L. pennellii*. However, they contained only a portion of the mitochondrial genome of the donor species, whereas *L. pennellii* chloroplasts were actually transferred in experiments with asymmetric and symmetric hybrids (Bonnema and O'Connel 1992a). The development of nuclear-cytoplasm incompatibility was considered to be one cause of the impossibility to transfer the plastome of the donor species during the construction of cybrids on the one hand and the morphologic anomalies in such cybrids on the other hand (Bonnema et al. 1991, 1995). Cybrids of cultivated tomato with chloroplasts of *L. peruvianum* var '*dentatum*' obtained in our laboratory (Ratushnyak et al. 1991), however, were male-sterile. In addition alloplasmatic incompatibility of these plants resulted in partial chlorophyll deficiency (marbled variegation and light-green leaves) and stunted growth and development (Ratushnyak et al. 1995). Structural and functional breaches in the photosynthetic apparatus of cybrid plants *Lycopersicon esculentum*(+*L. peruvianum* var '*dentatum*') also proved plastome-genome incompatibility (Kochevenko et al. 1999).

Here we for the first time our succes in obtaining fertile cybrid plants with the reverse alloplasmatic structure (the nuclear genome of *L. peruvianum* var '*dentatum*' and chloroplasts of *L. esculentum*). The cybrids obtained were characterized on the basis of chromosome numbers, isozyme and RAPD patterns, morphology, fertility and by RFLP analysis of organelle DNAs. We also propose to use the assessment of the photosynthetic apparatus state as a criterium in evaluating the incompatibility of the nuclear and chloroplast genomes in cybrids.

Materials and methods

Plant material

Seeds of cultivated tomato *Lycopersicon esculentum* Mill. (cv 'Quedlinburger Frühe Liebe', 2n = 2x = 24) were kindly supplied by Dr. C. Lehmann (Institute of Genetics and Crop Plant Research, Gatersleben, Germany). In vitro-grown plants of the plastome chlorophyll-deficient mutant Lp-alb3 of *L. peruvianum* var '*dentatum*' Dun. (line 3767, 2n = 2x = 24) were obtained from Dr. V. Rudas (Institute of Cell Biology and Genetic Engineering, Kiev, Ukraine). Axenic shoots of Lp-alb3 were cultivated on the hormone-free medium P (Sidorov et al. 1981) supplemented with 30 g/l of sucrose at 25° ± 1 °C, 3000 lux illumination and a 16-h photoperiod. Seeds of wild tomato *L. peruvianum* var '*dentatum*' Dun. (line 3767) were kindly provided by Drs. A. Zhuchenko and N. Bocharnikova (Institute of Genetics, Kishinev, Moldova). The seeds were sterilized and germinated as previously described (Kochevenko et al. 1996). The plants were propagated in vitro on a TM-1 medium (Shahin 1985).

Isolation and fusion of protoplasts

Leaves of aseptically cultured plants were used as the source of protoplasts. The composition of the maceration mixture and the procedure for protoplast isolation have been described previously (Kochevenko et al. 1996). Before fusion the donor protoplasts (*L. esculentum*) were inactivated by γ-irradiation (1000 Gy, [^{60}Co] source, 600 Gy/h). Protoplast fusion was carried out as described by Menczel et al. (1981).

Protoplast culture and plant regeneration

After the fusion protoplasts were cultured at initial density of 1×10^6/ml in KM8p medium (Kao and Michayluk 1975). The cultures were incubated at 25 °C in the dark for the first 5 days and then exposed to the light. Small colonies that were present after 3 weeks of culture were transferred to TM-3 medium. Minicalli formed on TM-3 agar solidified medium were transferred to TM-4 regeneration medium. The hybrid lines were identified by their green colouration. The regenerating shoots were rooted on hormone-free MS (Murashige and Skoog 1962) medium.

Cytological analysis

Chromosome counts were made on root tips of in vitro growing plants as described by Kochevenko et al. (1996).

Isozyme analysis

Proteins were isolated from young leaves in buffer solution containing 0.05 *M* tris, 0.01 *M* β-mercaptoethanol, 12% glycerol,

pH = 7.5 (leaf: buffer – 1:2). Electrophoretic separation of the proteins was carried out on 10% polyacrylamide gels. A veronal electrode buffer was used (5.5 g/l veronal, 1 g/l tris). For visualization of multiple molecular forms of esterase (EST) the gels were treated according to Brewer (1970). Peroxidase (PER) activity was visualized using a saturated benzidine solution in the presence of 1% H_2O_2.

Morphology and fertility

In order to study fertility and morphology we transplanted the cybrids into the open field. Individual plants were evaluated for bush size, leaf morphology, inflorescence structure and fruit shape. The fertility of the cybrids was determined by two criteria: (1) male fertility based on stainability and pollen germination on a solidified medium as described by Ratushnyak et al. (1995); (2) female fertility based on the crossability of cybrids as seed parents with *L. peruvianum* var '*dentatum*' (line 3767) as pollinators.

Analysis of organelle and nuclear DNAs

Chloroplast and mitochondrial DNA (cp- and mtDNA) were isolated from aseptic plants according to Bookjans et al. (1984) and Wilson and Chourey (1984), respectively. MtDNA was digested with *Bam*HI, *Eco*RV and *Hin*dIII restriction enzymes, and cpDNA was cleaved with *Hin*dIII and *Hpa*-II. The fragments were separated on 0,8% agarose and stained with ethidium bromide.

Total DNA was extracted from leaves of the in vitro-grown plants as described by Edwards et al. (1991) and was analyzed by polymerase chain reaction (PCR) amplification with random primer OPA-09 (Operon Technologies, Alameda, Calif.). Polymerase chain reactions were carried out in 25-μl volumes containing 20 ng of genomic DNA, 1 U *Tag* DNA polymerase (Promega) and 1× reaction buffer (Promega), 0.2 m*M* of each dNTP, 1.5 m*M* $MgCl_2$ and 0.25 μ*M* random primer, topped off with mineral oil. The thermal cycles used were: 1 cycle of 3 min at 94 °C, 0.5 min at 36 °C and 1 min at 72 °C, followed by 33 cycles of 0.3 min at 94 °C, 0.5 min at 36 °C and 1 min at 72 °C; and a final stage of 10 min at 72 °C. The amplification products were electrophoresed in 1% agarose gels and visualized by staining with ethidium bromide.

Study of pigment content

The pigments were extracted from 1 g of fresh tissue by 100% acetone. Pigment contents were determined spectrophotometrically according to Lichtenthaler et al. (1987).

Measurement of chlorophyll fluorescence induction

The fluorescence induction curves of leaves were recorded with a one-beam installation assembled in the laboratory. Fluorescence measured at 685 nm was excited by blue radiation of a mercury arc. The time necessary for full opening of a shutter equaled 3 ms. The actinic light intensity at the level of the leaf blade was 25 W/m^2. The leaves were kept for 15 min in the dark before recording.

CO_2 exchange

The intensity of CO_2 assimilation was measured by an infrared gas analyser OA-5501. Measurements were conducted on intact attached leaves at a temperature of 25 °C and illumination of 400 W/m^2 at the level of a leaf. An incandescent lamp KG 220–1000 was used as the source of light with a heliofilter consisting of 0.5% $CuCl_2$ solution. Calculations were performed for 1 g of dry matter.

Analysis of thylakoid membrane components

Thylakoids were isolated and purified as described by Machold et al. (1979). The pigment proteins of the thylakoid membranes were separated by mild dissociating SDS-PAGE according to the procedure of Anderson (1980). After electrophoresis the pattern of the chlorophyll-protein complexes was scanned by a densitometer at 677 nm. Pigment-protein zones were calculated as a percentage of all peaks. Thylakoid membrane polypeptides were analysed on slab 10–20% gradient polyacrylamide gels as described by Laemmli (1970). Gels were stained in Coomassie Blue R-250.

Results

Protoplast culture and plant regeneration

A Mixture of mesophyll protoplasts of *L. esculentum*, *L. peruvianum* var '*dentatum*' and products of their fusion was cultivated in the KM8p medium. The first cell devisions were observed on the 7th day. Plating efficiency was 30%. Cell colonies were transported to the agar-solidified medium, firstly to TM-3 and then to TM-4. Hybrid selection was performed at the mini-callus stage. Since protoplasts of *L. esculentum* were unable to undergo cell division following the γ-irradiation and protoplasts of the Lp-alb3 mutant formed white colonies, all colonies able to synthesize chlorophyll were determined to be hybrid ones. In total, 190 green colonies were selected. The TM-4 medium was used to induce organogenesis. The regeneration index was 38%. We selected three to four regenerants from each green colony in order to study possible ways of segregating their cytoplasmic organelles and reconstructing their DNA.

Selection and biochemical analysis of cybrids

Preliminary screening of the cybrids among the selected regenerants was performed by cytogenetic analysis. The search for regenerated plants with a chromosome number of 2n = 2x = 24 was carried out. Most of the plants had a tetraploid set of chromosomes (2n = 4x = 48). While certain portion of the regenerants had an aneuploid set of chromosomes (2n = 4x − 2 = 46 or 2n = 4x − 1 = 47). We found 24 chromosomes (2n = 2x = 24, Fig. 1) in cells of just three subclones, B1A, B3A and B4A, regenerated from a single colony. These were used for further morphological, backcrossing and biochemical analyses. All subclones had *peruvianum*-specific bands of peroxidase and esterase and randomly amplified polymorphic DNA (RAPD) patterns similar to that of *L. peruvianum* var '*dentatum*' (Figs. 2, 6; Table 2). *Hpa*II and *Hin*dIII restriction enzyme patterns of cpDNA of the three subclones and of *L. esculentum* were identical (Fig. 3) which proved not only the inheritance of cultivated tomato chloroplasts but also their cybrid origin. To characterize the chondriome composition of the cybrid plants mtDNA

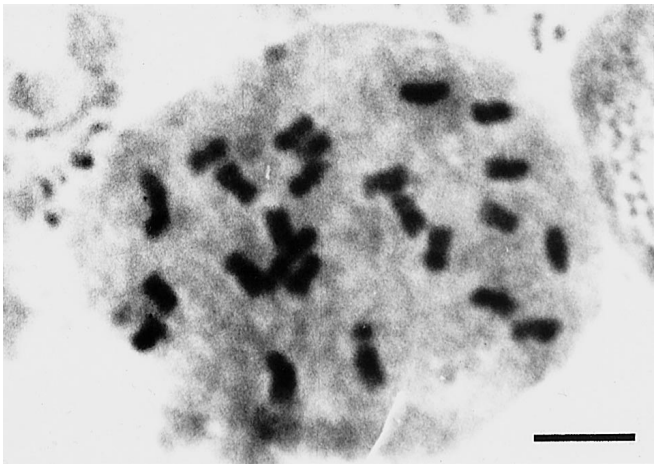

Fig. 1 Metaphase plate of cybrid *L. peruvianum* var '*dentatum*' (+*L. esculentum*) subclone B1A (2n=2x=24). Bar:13 μm

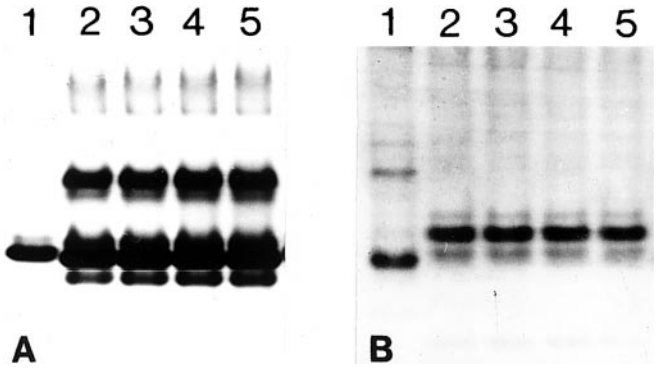

Fig. 2 Peroxidase (**A**) and esterase (**B**) isoenzyme patterns of *L. esculentum* (*lane 1*),*L. peruvianum* var '*dentatum*' (*2*) and cybrid subclones B1A, B3A, B4A (*3, 4, 5,* respectively)

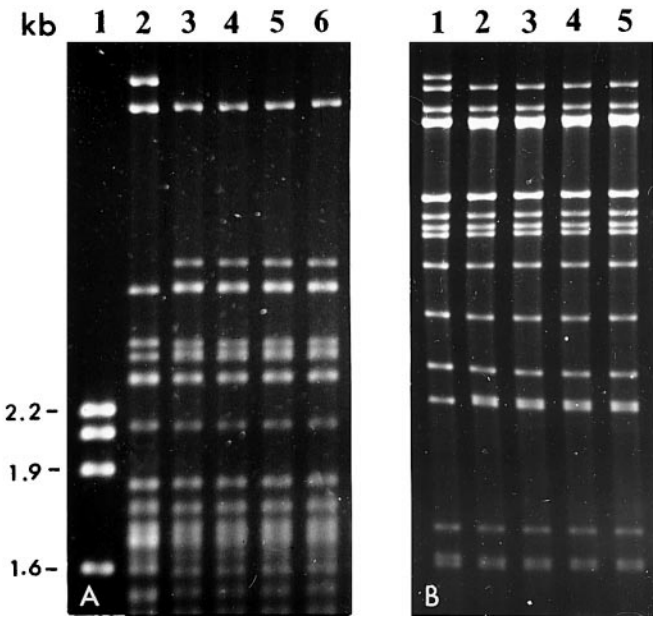

Fig. 3A,B Restriction enzyme analysis of cpDNA of cybrid plants and their parents. **A** cpDNA was digested with*Hpa*II: *lanes 1* lambda *Hpa*II fragments, *2 L. peruvianum* var '*dentatum*', *3 L. esculentum, 4, 5, 6* cybrid subclones B1A, B3A and B4A respectively. **B** cpDNA was digested with *Hin*dIII. *lanes 1 L. peruvianum* var '*dentatum*', *2 L. esculentum,* (3, 4, 5) cybrid subclones B1A, B3A and B4A, respectively

was digested with *Eco*RV, *Hin*dIII and *Bam*HI restriction endonucleases. All of these enzymes produced different restriction patterns between parents and cybrid plants, while differences in patterns among subclones was not found. Restriction patterns of subclones B1A, B3A and B4A, apart from *L. esculentum*-specific fragments, also had the fragments specific only for *L. peruvianum* var '*dentatum*'. In addition, a new non-parental *Bam*HI fragment was observed (Fig. 4).

Morphology and fertility

Cybrid plants were characterized by a high viability, and they produced fully developed flowers and formed fruits with viable seeds. They fully resembled the *L. peruvianum* var '*dentatum*' on the basis of basic morphological traits of their bushes, stems, leaves, flowers and fruits (Table 1, Fig. 5). The frequency of fruit formation in the cybrid subclones after their pollination with *L. peruvianum* var '*dentatum*' pollen was high (Table 2). Pollen fertility of all cybrid plants and especially of the subclone B4A was lower than that of *L. peruvianum* var '*dentatum*' (Table 2). Plants of the R1 progeny were developed by backcrossing cybrid subclones (Ro) with the peruvian tomato. Their morphology was identical to that of *L. peruvianum* var '*dentatum*'. While their pollen fertility (germination was about 10–12%) was comparable with that of the maternal Ro plants.

Analysis of the photosynthetic apparatus

No significant decrease in the general content of Chl (a+b) was observed in the leaves of the cybrid plants and their sexual progenies in comparison with parental forms (Table 3). The ratios of Chl a/b and Chl (a+b)/Car (x+c) were also practically identical (data not shown).

In order to determine any possible breaches in light reactions of photosynthesis we studied the chlorophyll fluorescence induction. The fluorescence induction curves in both cybrid plants *L. peruvianum* var '*dentatum*' (+*L. esculentum*) and the parental forms were practically identical. The Fv/Fp ratio, which characterizes photochemical activity of PS II in the cybrid plants and *L. peruvianum* var '*dentatum*' was 0.71 and 0.72, respectively. The cybrid *L. peruvianum* var '*dentatum*' (+*L. esculentum*) also had a low (Fi-Fo)/Fv ratio used for evaluation of the quantity of reaction

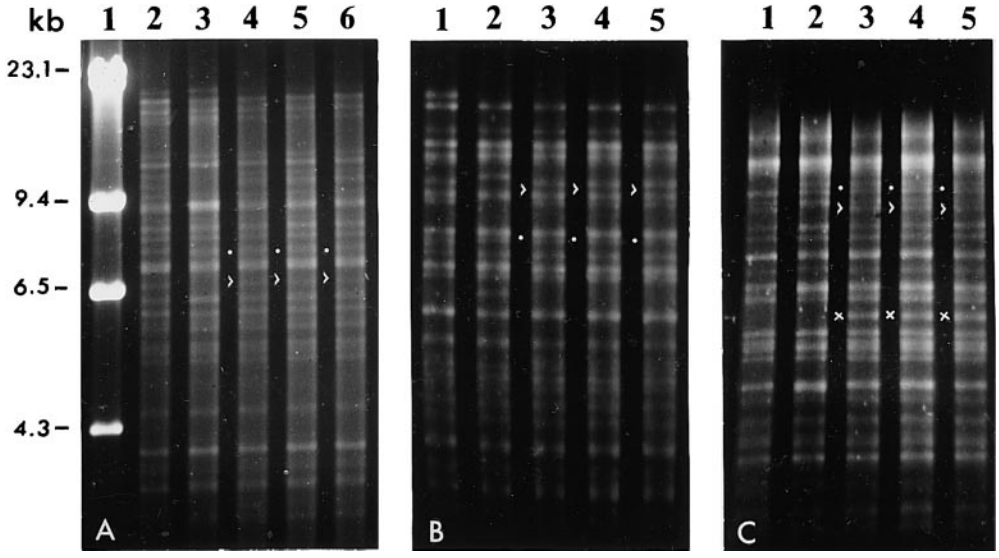

Fig. 4A–C Mitochondrial genome analysis. **A** MtDNA was digested with *Eco*RV: *lanes 1* lambda *Hin*dIII fragments, *2 L. peruvianum* var '*dentatum*', *3 L. esculentum, 4, 5, 6* cybrid subclones B1A, B3A and B4A, **B, C** MtDNA samples were digested with *Hin*dIII and *Bam*HI respectively: *lanes 1 L. peruvianum* var '*dentatum*', *2 L. esculentum, 3, 4, 5* cybrid subclones B1A, B3A and B4A, Arrowhead indicate *L. peruvianum* var '*dentatum*'-specific fragments, *L. esculentum*-specific fragments are marked by dots. × represents a new cybrid -specific fragment

centres which are inactive in non-cyclic electron transport. A study of the intensity of CO_2 assimilation was carried out to assess the efficiency of the dark reactions of photosynthesis. The intensity of photosynthesis for the cybrid of *peruvianum* tomato was 32.5% higher than that of *L. esculentum* and 9.6% lower than that of *L. peruvianum* var '*dentatum*' (Table 3).

Mild electrophoresis on a polyacrylamide gel was perfomed for the analysis of pigment-protein complexes PS I and PS II. Densitometric scanning of green pigment-associated protein profiles showed that the relative total amount of the light-harvesting complex PS II ($LHCP^1 + LHCP^3$) in the cybrid subclones only slightly varied from that of the parental species. There were also no noticeable differences in relative content of the reaction centre complexes PS II and PS I (Table 3). The analysis of the thylakoid membrane polypeptides spectra did not show any qualitative or quantitative differences between the cybrid plants and initial species (data not shown).

Discussion

We have used a plastome chlorophyll-deficient mutant as a recipient and strongly γ-irradiated donor species in our experiments on protoplasts fusion. The selection of presumable cybrids was conducted based on the restored ability of hybrid colonies to biosynthesize chlorophyll this selection system has been shown to be effective in obtaining cybrids in the genus *Lycopersicon* (Ratushnyak et al. 1991; Derks et al. 1992).

Cytogenetic and isozyme analyses have shown that all regenerated plants with the exception of the subclones (B1A, B3A and B4A) were nuclear somatic hybrids (symmetric or asymmetric). This fact is considered quite interesting because in previous experiments of Bonnema et al. (1991) and Derks et al. (1992) the doses of 1000 and 300 Gy ensured the complete elimination of the nuclear genome of *L. pennellii* and *L. hirsutum* in obtaining cybrids of the cultivated tomato.

Table 1 Morphological peculiarities of the stem, leaves, flowers and fruits of *L. esculentum*, *L. peruvianum* var '*dentatum*' and their cybrid plants

Genotype	Stem		Leaf		Flower		Fruit	
	Height (mm)	Hairiness	Surface type	Colour	Hairiness of sepals	Pistil position	Weight (g)	Colour
Lycopersicon esculentum cv 'Quedlinburger Frühe Liebe'	High (110)	Thickly haired	Highly goffered	Dark-green	Thickly haired	Shorter than stamens	Small or medium (30–60)	Red
L. peruvianum var '*dentatum*' line 3767	High (130)	Hairless	Smooth	Dark-green	Hairless	Longer than stamens	Very small (3–4)	Green
Cybrid *L. peruvianum* var '*dentatum*' (+*L. esculentum*) subclone B1A	High (120)	Hairless	Smooth	Dark-green	Hairless	Longer than stamens	Very small (3–4)	Green
Sexual progeny of cybrid plants line F1A-2	High (130)	Hairless	Smooth	Dark-green	Hairless	Longer than stamens	Very small (3–4)	Green

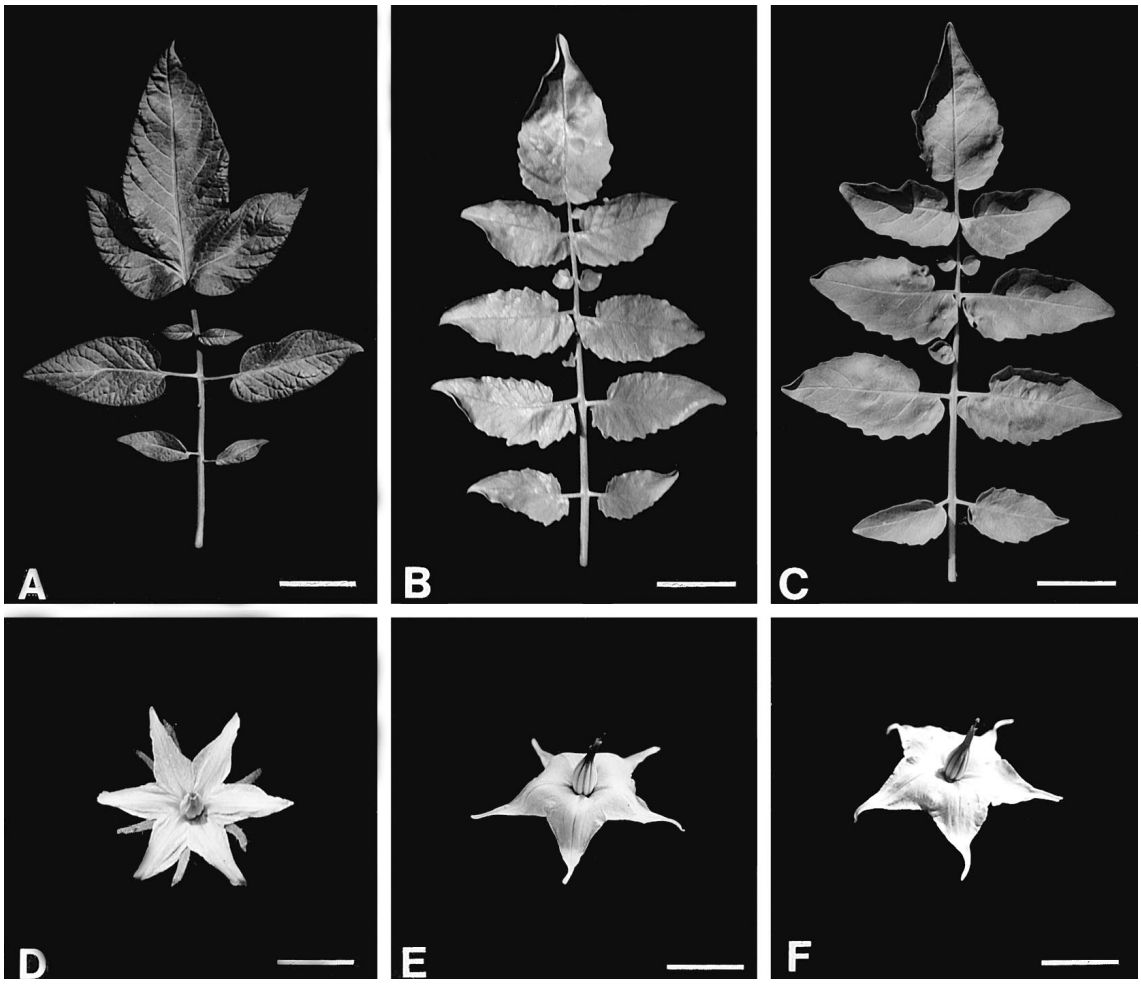

Fig. 5A–F Comparative morphology of leaves and flowers of parental species and cybrid plants. **A, D** *L. esculentum* cv 'Quedlinburger Frühe Liebe', **B, E** cybrid *L. peruvianum* var '*dentatum*' (+*L. esculentum*) subclone B1A, **C, F** *L. peruvianum* var '*dentatum*'. Bars: 29 mm (**A**), 25 mm (**B**), 26 mm (**C**), 11 mm (**D**), 13 mm (**E, F**)

We managed to obtain cybrid plants *L. esculentum* (+*L. peruvianum* var '*dentatum*') using a dose of 200 Gy only (Ratushnyak et al. 1991). At the same time Derks et al. (1991) used the dose of 1000 Gy for producing asymmetric hybrids between *L. peruvianum* and *L. esculentum.* The above-mentioned protocol assumes that γ-irradiation is necessary only for the inactivation of protoplast divisions in a donor plant, while the process of cybrid formation is probably dependent on the random segregation of the donor nucleus in heterokaryocyte developed after the fusion of two protoplasts.

Cytogenetic, morphological and biochemical analyses of plants B1A, B3A and B4A proved that they were cytoplasmic hybrids of *peruvianum* tomato with the plastome of *L. esculentum* and reconstructed chondriome (Figs. 1–6, Tables 1, 2). Our analysis of the morphology and fertility of independent lines of the sexual progeny R1 that was developed through backcrossing these subclones with *L. peruvianum* var '*dentatum*' (Tables 1, 2) also supports this conclusion. Rearrangements of mtDNA probably occurred as a result of the mtDNA of both cultivated and *peruvianum* tomatoes recombining. Restriction patterns of mtDNA of cybrid plants contain not only species-specific *Bam*HI, *Eco*RV and *Hin*dIII fragments of both fusion partners but also the new discovered *Bam*HI-fragment that was observed solely in the mt-DNA of cybrid plants. This excludes the possibility of cybrid plants inheriting mitochondria of two species because they lack all the other specific *Bam*HI and *Hin*d III fragments of the cultivated and *peruvianum* tomatoes (Fig. 4).

To explain why all the subclones had the reconstructed chondriome and not a chondriome of one of the parents is, however, more complicated. The presence of recombined or parental mtDNA can be the result of the random segregation of mitochondria in a fusion product. While mtDNA recombination is a non-random process, it is a vital condition for a cybrid cell to function as a whole and balanced genetic system. As already known, uniparental inheritance of plasmagenes after sexual hybridization is a characteristic for most

Table 2 Cytogenetic and biochemical characteristics of *L. esculentum*, *L. peruvianum* var '*dentatum*' and cybrid plants *L. peruvianum* var '*dentatum* (+*L. esculentum*) and their sexual progeny

Genotype	Number of chromosomes	Fruit formation after pollination with *L. peruvianum* var '*dentatum*' line 3767 pollen (% ± SD)	Pollen fertility		Biochemical analysis						
			Staining (% ± SD)	Germination (% ± SD)	Isozymes[a]		RAPD patterns	cpDNA		MtDNA		
					EST	PRX		*Hin*dIII	*Hpa*II	*Hin*dIII	*Bam*HI	*Eco*RV
Lycopersicon esculentum cv 'Quedlinburger Frühe Liebe'	24	0	96.6 ± 2.7	32.6 ± 2.1	E[b]	E	E	E	E	E	E	E
L. peruvianum var '*dentatum*' line 3767	24	90 ± 2.0	97.7 ± 3.1	30.4 ± 1.5	P	P	P	P	P	P	P	P
Cybrid subclone B1A	24	88 ± 2.1	93.5 ± 2.3	12.3 ± 1.3	P	P	P	E	E	R	R	R
Cybrid subclone B3A	24	90 ± 1.7	92.0 ± 2.9	8.1 ± 2.7	P	P	P	E	E	R	R	R
Cybrid subclone B4A	24	85 ± 2.3	79.4 ± 3.5	6.8 ± 2.0	P	P	P	E	E	R	R	R
Sexual progeny of cybrid plants line F1A-2	24	86 ± 3.1	91.1 ± 2.0	9.7 ± 2.1	P	P	P	E	E	nd	nd	nd

[a] Est, Esterase; Prx, Peroxidase
[b] E, *L. esculentum* type; P, *L. peruvianum* var '*dentatum*' type; R, rearranged type; nd, not determined

plant species. That is why for the normal functioning of chloroplasts it is essential to have a certain chondriome type which is determined from the coevolution of genetic cell compartments. Mitochondria genome rearrangements resulting from spontaneous mutations (Roussell et al. 1991) and/or cell-engineering manipulations with protoplasts (Bonnema et al. 1995) can lead to disturbances in chloroplast development. Moreover, the unselective co-transfer of mitochondria of a donor-species together with the selective transfer of chloroplasts or a partial co-transfer of the donor chondriome by means of mtDNA recombination took place in experiments on somatic hybridization obtaining cybrids between phylogenetically remote species (Menczel et al. 1983; Medgyesy et al. 1985; Zubko et al. 1995). *L. esculentum* and *L. peruvianum* var '*dentatum*' are the most phylogenetically remote species in the genus *Lycopersicon* (Rick et al. 1990; Palmer and Zamir 1982; McClean and Hanson 1986). That is why their mtDNA recombination might be necessary for optimal nuclear-mitochondrial and chloroplast-mitochondrial interrelations in cybrid subclones of the *peruvianum* tomato with the chloroplasts of *L. esculentum.* Cybrid plants of the cultivated tomato with the chloroplasts of *L. peruvianum* var '*dentatum*' and reconstructed chondriome which we obtained earlier had some new phenotypic traits (stunted growth and development, marbled variegation and light green leaves and partial male fertility), unlike the parental species (Ratushnyak et al. 1995), and structure-functional damages in the photosynthetic apparatus (Kochevenko et al. 1999). This testified to the presence of alloplasmic incompatibility of genetic compartments in this combination. It was interesting to observe whether a combination of nuclear genome of *L. peruvianum* var '*dentatum*' and plasmagenes of *L. esculentum* would cause damaging nuclear-cytoplasmic interactions visible in the form of morphological and physiological abnormalities in the cybrids with the reverse alloplasmic organization. *L. peruvianum* var '*dentatum*' (+*L. esculentum*). The detailed morphological analysis of the cybrid plants showed that all the subclones were identical to *L. peruvianum* var '*dentatum*' based on the morphological traits of bushes, stems, leaves, flowers and fruits (Table 1, Fig. 5). A lower pollen fertility of subclones B1A, B3A and B4A is a very interesting fact (Table 2). At the same time fruit formation in the cybrid plants and *peruvianum* tomato pollinated by *L. peruvianum* var '*dentatum*' pollen was 85–90%. It is well known that abnormalities in pollen development in cybrid plants appear as a result of a disturbed coordination in the interactions between the nuclear and mitochondrial genomes (Izhar and Zelcer 1986 and references therein). Such abnormalities can develop as a result of either mtDNA rearrangement or nuclear-mitochondrial incompatibility (Kofer et al. 1991; Melchers et al. 1992; Breiman and Galun 1990). On the other hand, the presence of some donor mtDNA does not always cause complete male sterility but results in decrease in pollen fertility

Table 3 Analysis of chlorophylls' content, parameters of the chlorophyll fluorescence induction curves, CO_2-exchange and pigment-protein complexes of cybrid plants and parental forms. Data represent the mean values ± SE of three independent experiments (nd, not determined)

Genotype	Content Chl (a + b) mg/g fresh weight	Parameters of the chlorophyll fluorescence[a] induction curves		Photosynthesis intensity μg CO_2/g (dm) s	Relative content of pigment-protein complexes[b] (%)		
		Fv/Fp	(Fi-Fo)/Fv		LHCP[1] + LHCP[3]	CP1a + CP1	CPa
Lycopersicon esculentum cv 'Quedlinburger Frühe Liebe'	2.99 ± 0.12[c]	0.74 ± 0.01[a]	0.35 ± 0.04[a]	16.8 ± 1.2[a]	67.1 ± 1.0[a]	19.4 ± 0.7[a]	19.6 ± 0.3[a]
L. peruvianum var '*dentatum*' line 3767	3.21 ± 0.06[b]	0.72 ± 0.01[a]	0.31 ± 0.02[a]	27.3 ± 0.9[b]	66.9 ± 0.6[a]	16.6 ± 0.6[b]	16.4 ± 0.9[b]
Cybrid *L. peruvianum* var '*dentatum*' (+ *L. esculentum*) subclone B1A	3.50 ± 0.09[b]	0.71 ± 0.01[a]	0.33 ± 0.03[a]	24.9 ± 2.2[b]	68.7 ± 0.8[a]	15.8 ± 0.6[b]	15.7 ± 0.6[b]
Sexual progeny of cybrid plants line F1A-2	3.37 ± 0.06[b]	0.71 ± 0.02[a]	0.35 ± 0.02[a]	23.0 ± 1.7[b]	nd	nd	nd

[a] Fo, Fp, Initial and maximal fluorescence levels; F_i, fluorescence level at plateau; Ft, fluorescence level after a 6-min illumination, Fv, variable fluorescence
[b] CP1a and CP1, Reaction centre complex of PS 1; CPa, reaction centre complex of PS 2; LHCP[1] and LHCP[3], different molecular weight forms of LHC-II
[c] Means within a column followed by the same letter are not significantly different at P = 0.05

(Tanno-Suenaga et al. 1988; Bonnema et al. 1991). Since the plants of all subclones (B1A, B3A and B4A) were characterized with a lower male fertility we suppose that rearrangements in their mtDNA probably result in changes in nuclear-mitochondrial interactions that are phenotypically manifested in a decrease in the ability to produce functional pollen.

A study of the photosynthetic apparatus in the cybrids *L. peruvianum* var '*dentatum*' (+ *L. esculentum*) was the next step in the elucidation of interactions between the nuclear genome of *L. peruvianum* var '*dentatum*' and plasmagenes of *L. esculentum*. The photosynthetic apparatus was chosen as the object of our investigation because of two reasons. First, the structural features of chloroplasts and, subsequently their functionality are determined by genes that are localized in the nuclear genome and plastome (Herrmann 1989). Second, investigations of sexual as well as cytoplasmic hybrids indicated that rearrangements of the mitochondrial genome could also induce an abnormal structural organization and functional activity of chloroplasts (Roussell et al. 1991; Gu et al. 1993; Bonnema et al. 1995). Hence, it would be logic to expect breaches in organization and function of the photosynthetic apparatus in cybrids if they have disturbances in the nuclear-chloroplast or/and chloroplast-mitochondrial interactions. Dark-green coloured leaves and a high viability (pronounced growth and fruit-bearing capabilities) of symmetric somatic hybrids *L. esculentum* + *L. peruvianum* that carry the chloroplast genome of the cultivated tomato indicate the normal functioning of *L. esculentum* chloroplasts against the background of the hybrid genome (San et al. 1990; Wijbrandi et al. 1990b; Derks et al. 1991). This conclusion was proven directly in investigations of progeny of somatic hybrids *L. esculentum* + *L. peruvianum*, which are characterized by the analogous genetic constitution. Analyses of gross photosynthesis rate, chlorophyll content and chlorophyll fluorescence revealed a high photosynthetic capacity in these plants (Bruggemann et al. 1995). At the same time the absence of nuclear genetic material of the *peruvian* tomato in cybrids of the cultivated tomato carrying the plastome of *L. peruvianum* induced a decrease in photosynthetic activity as a result of a disruption of structure and malfunction of chloroplasts (Kochevenko et al. 1999). Reconstruction of the cytoplasm in cybrid plants could lead to qualita-

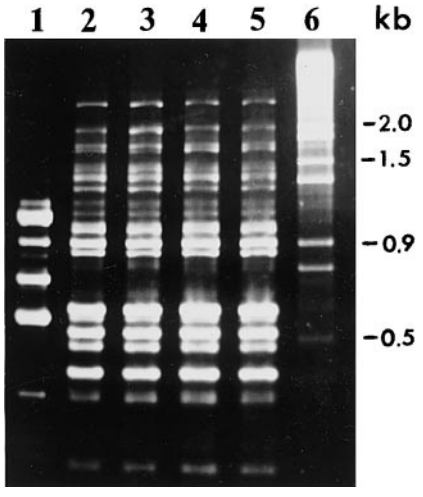

Fig. 6 RAPD patterns of cybrid plants and their parents generated using primer OPA-09. *Lanes 1 L. esculentum, 2 L. peruvianum* var '*dentatum*', *(3, 4, 5)* cybrid subclones B1A, B3A and B4A, respectively and *6* molecular-weight markers (λDNA digested with *Hin*dIII and *Eco*RI

tive as well as quantitative changes in the composition of the photosynthetic pigments (Kushnir et al. 1991; Perl et al. 1991; Zubko et al. 1994). The spectrophotometric analysis, however, did not show any significant quantitative differences in the content of the main photosynthetic pigments between cybrids of *peruvianum* tomato and the initial parental species. Plants characterized by abnormal pigmentation were not found in R1 progeny of these cybrid plants. The average chlorophyll content was higher in these plants than in *L. esculentum* and higher than or the same as in *L. peruvianum* var '*dentatum*' (Table 3). No significant deviations in the content of reaction centre complexes PS II and PS I and light-harvesting complex PS II were discovered during the analysis of pigment-protein content of thylakoid membranes of cybrid plants (Table 3). However, according to the data of Babiychuk et al. (1995) plastome-genome incompatibility in the cybrid plants of *Nicotiana tabacum* (+*Atropa belladonna*) was expressed by changes in the LHCP II polypeptides. That is why we carried out the electrophoretic analysis of polypeptides in the chloroplast thylakoid membranes of the *peruvianum*-tomato cybrids. Neither a significant drop in the content nor the appearance of additional polypeptides in the light-harvesting complex PS II were found. Therefore, violations in the structural organization of pigment-protein apparatus are not a characteristic of the cybrids *L. peruvianum* var '*dentatum*' (+*L. esculentum*). Our results on fluorescence induction and CO_2 assimilation testify to a high functional activity of the photosynthetic apparatus in the cybrid plants of the *peruvianum*-tomato and their sexual progeny (Table 3). Since investigations of cybrid plants *L. peruvianum* var '*dentatum*' (+*L. esculentum*) and their sexual progeny reveal neither vegetative and reproductive developmental abnormalities nor structure-functional changes in the photosynthetic apparatus, we can conclude that the *L. esculentum* plastome can effectively interract with the nuclear genome of *L. peruvianum* var '*dentatum*'.

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