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Limited DNA elimination from the irradiated potato parent in fusion products of albino *Lycopersicon esculentum* and *Solanum tuberosum*

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Summary. This paper describes the analysis of the elimination of potato DNA from potato-tomato somatic cell hybrids. The hybrids were obtained by fusion of protoplasts of a cytoplasmic albino tomato genotype with leaf mesophyll protoplasts of a potato genotype carrying the β-glucuronidase (GUS) gene of *Escherichia coli*. The potato protoplasts were either isolated from unirradiated plants or from plants irradiated with 50 or 500 Gy of γ-rays. Green calli were selected as putative fusion products. The hybridity of these calli was confirmed by isoenzyme analysis. All of the green calli tested contained a potato-specific chloroplast DNA restriction fragment, and most of the calli analysed were positive for β-glucuronidase activity. In 72 of the hybrid calli we determined the percentage of potato nuclear DNA using species-specific probes. All of the tested green calli contained a considerable amount of potato genomic DNA, irrespective of the dose of irradiation of the potato parent. The limited degree of potato DNA elimination and the absence of true cybrids are discussed.

Key words: Tomato – Potato – Asymmetric somatic hybrids – Gamma irradiation – Plastome-genome interaction

Introduction

For the improvement of cultivated plant species it is often desirable to add one or a few favourable traits from another more or less related species. If the trait concerned is present in a species that cannot be hybridized sexually with the crop plant, the desired gene(s) can be combined with the genome of the recipient by means of somatic hybridization. Since the donor will contain not only the trait of interest but also many unfavourable genes, in most cases the transfer of only a small part of the donor genome to the recipient species is intended.

One way to achieve partial genome transfer is to irradiate the donor species with a high dose of X- or gamma-rays. Several investigators have used this strategy. In some cases, only one or a few donor chromosomes were found to be retained within the hybrid (Bates et al. 1987; Dudits et al. 1980; Gupta et al. 1984), whereas in others, elimination of the irradiated genome appeared to be limited (Famelaer et al. 1989; Imamura et al. 1987; Müller-Gensert and Schieder 1987; Wijbrandi et al. 1990; Yamashita et al. 1989). In all these studies, the fusion products were selected on the basis of nuclear-encoded traits of both parents; therefore, the limited elimination of the donor genome may be explained in part by "dragging" of nuclear DNA by the genes for which selection was carried out.

To investigate quantitatively the elimination of genetic material from the irradiated donor species without any bias for a selected or dragged nuclear-encoded trait, experiments were performed in which the isolation of the 'hybrid' calli was based on a cytoplasmically controlled trait of the donor species. For this purpose protoplasts derived from suspension cultures of *Lycopersicon esculentum*, genotype ALRC, which has a cytoplasmically inherited albino mutation, were fused with leaf protoplasts of *Solanum tuberosum* plants that had been irradiated with a low (50 Gy) or high (500 Gy) dose of gamma-rays, or left unirradiated. Selection was based on the transfer of functinal chloroplasts from potato to the albino tomato, i.e. green calli were selected. The amount of potato nuclear DNA in the obtained fusion calli was determined in dot blot analyses (Saul and Potrykus 1984)

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by means of probes recognizing species-specific repetitive DNA sequences.

Tomato and potato are plant species that cannot be hybridized sexually, but somatic hybrids between both species have been obtained (Melchers et al. 1978; Shephard et al. 1983). This indicates that somatic incongruity does not prevent the formation of viable hybrid plants of these species.

Materials and methods

Plant materials

As recipient the maternally inherited albino mutant of *Lycopersicon esculentum* cv 'Large Red Cherry' (ALRC) (originating from Dr. M. R. Hanson, Section of Genetics and Development, Cornell University, Ithaca, USA (Hosticka and Hanson 1984)) was used. Callus cultures of this genotype were initiated on solid UM2D medium (the "callus and cell culture medium" of Uchimiya and Murashige 1974). Subsequently, cell suspensions were established by culturing callus tissue in liquid UM2D medium at 100 rpm on a gyratory shaker at a light intensity of 0.25 W/m^2 (16 h) at 25 °C. These cell suspensions were diluted 1:5 with fresh UM2D medium every 7 days. When measured by flow cytometry the ALRC suspension consisted of a mixture of cells with a 4C (16%), 8C (58%) or 16C (26%) DNA content (results not shown).

The donor species used was *Solanum tuberosum* 7322-K1. This genotype was obtained by transforming monoploid H7322 (originating from Prof. Dr. G. Wenzel, Grünbach, Germany (De Vries et al. 1987)) with an *Agrobacterium tumefaciens* strain containing a plasmid with a kanamycin-resistance (NPTII) gene and a β-glucuronidase (GUS) gene on one T-DNA. Apparently polyploidization had occurred during transformation/tissue culture, since the regenerated transformed plant was tetraploid. Shoots of this genotype were subcultured aseptically every 4 weeks in plastic containers on medium containing MS salts (Murashige and Skoog 1962), vitamins according to Tewes et al. (1984) and 20 g/l sucrose, supplemented with 150 mg/l kanamycin and solidified with 8 g/l agar, and grown at a light intensity of 10 W/m^2 (Philips TLD 36 W; 16 h) at 25 °C.

Isolation, fusion and culture of protoplasts

For protoplast isolation of the ALRC genotype, 50 ml of a fast growing, 3- to 4-day old cell suspension culture was centrifuged for 5 min at 600 rpm (45 *g*). The pellet was resuspended in 30 ml AM medium (CPW salts (Frearson et al. 1973), 0.4 *M* mannitol, 10 m*M* $CaCl_2 \cdot 2H_2O$ and 100 mg/l 2[*N*-morpholino]-ethane-sulfonate (MES)), supplemented with 10 g/l Cellulase R10 and 2 g/l Macerozym R10 (both enzymes from Yakult, Japan), pH 5.6, and incubated for 16 h in the dark at 25 °C. The suspension was filtered through a 50-ml syringe containing glass wool into 10-ml plastic tubes and centrifuged for 5 min at 600 rpm (45 *g*). The pelleted protoplasts were resuspended in BM medium (0.4 *M* mannitol, 0.5 m*M* $CaCl_2 \cdot 2H_2O$, pH 5.8), centrifuged for 5 min at 600 rpm (45 *g*) and resuspended again in BM medium. A sample was counted in a haemocytometer to determine the concentration. The suspension was centrifuged once more and resuspended in BM medium to a final concentration of 0.5×10^6 protoplasts/ml.

Prior to protoplast isolation 7322-K1 plants were irradiated with 50 or 500 Gy of gamma-rays from a ^{60}Co source at a dose rate of approximately 2,000 Gy/h at the Pilot-Plant for Food Irradiation, Wageningen (the Netherlands). These plants were placed at 4 °C in the dark for 8 h, together with unirradiated 7322-K1 plants. Subsequently, the leaves (±1 g/petri dish) were cut in small pieces and incubated for 16 h in the dark at 25 °C in 12 ml AS medium (= AM medium with 0.4 *M* sucrose instead of mannitol) plus enzymes. The protoplasts were separated from cell debris by filtration through a nylon filter with a pore size of 50 µm and centrifuged for 10 min at 1,000 rpm (125 *g*). The floating protoplasts were rinsed once in AS medium without enzymes and twice in BS medium (0.4 *M* sucrose, 0.5 m*M* $CaCl_2 \cdot 2H_2O$, pH 5.8), after which the concentration was determined with a haemocytometer. After another centrifugation for 10 min at 1,000 rpm (125 *g*) BS medium was added to the protoplast layer to a final concentration of 0.5×10^6 protoplasts/ml.

Prior to electrofusion protoplasts from ALRC and 7322-K1 were mixed 1:1; 0.4 ml of this protoplast mixture was then transferred to a fusion chamber with five 2-mm-wide gaps between parallel stainless steel electrodes glued in a glass petri dish. The fusion apparatus was a combination of a function generator, a generator of direct current (DC) pulses and the fusion chamber. The electrofusion was performed as follows: the protoplasts were aligned in an alternating current (AC) field (1 MHz) of 100–150 V/cm; one or two DC pulses of 1,500 V/cm (20–30 µs) were applied, after which the AC field was slowly reduced to zero. The protoplast suspension was then transferred to a petri dish with an equal volume of culture medium in which all of the medium components, except glucose, were at double concentrations.

The protoplasts were cultured at a density of 2.5×10^5 protoplasts/ml in TM2G medium, which is modified TM-2 medium (Shahin 1985) containing 0.3 *M* glucose instead of 0.2 *M* sucrose, in the dark at 25 °C. When the first cell divisions were observed, mostly after 3–4 days, the cultures were diluted 1:1 with TM2G medium and exposed to dim light. After another 3–4 days the cultures were again diltued 1:1 in TM2G medium and solidified with 0.6% (final concentration) agarose (SeaPlaque, FMC BioProducts, USA). When microcalli were visible (after 3–4 days) the agarose was cut in pieces, transferred to a 9-cm diameter petri dish and diluted 1:1 with TMD medium (modified TM-2 medium containing 100 mg/l myo-inositol, 0.3 *M* sucrose and 0.1 mg/l NAA). Three to four days later, one-half volume TMD medium was added. Subsequently the liquid medium was replaced once a week with TMD medium until calli with a diameter of approximately 3 mm were obtained. Calli of this size were placed on solid medium: TMcµZ (modified TM-3 medium (Shahin 1985) containing 2.5 g/l sucrose, 36.4 g/l mannitol, 0.1 mg/l NAA instead of 2,4-D, 0.5 mg/l zeatin riboside instead of BAP) or TMcGZ (like TMcµZ, but containing 41 g/l glucose instead of sucrose and mannitol). Every 4 weeks the green calli were subcultured on new medium. Part of the calli was transferred to solid UM2D medium (the "callus and cell culture medium" of Uchimiya and Murashige 1974).

Chloroplast DNA analysis

Total DNA from leaves of ALRC and 7322 and from 13 putative fusion calli was isolated according to Dellaporta et al. (1983). After the last step NaCl was added to the DNA solution to a final concentration of 1 *M*, the solution was kept at −20 °C for 15 min and subsequently centrifuged at maximum speed in an Eppendorf centrifuge at 4 °C for 5 min. The DNA in the supernatant was ethanol precipitated, and the pellet was resuspended in TE buffer. Four micrograms of total DNA were digested with HinfI. DNA fragments were separated on a 1.2% agarose gel and transferred to Gene Screen Plus filter (Du Pont) as described by Derks et al. (1991). Hybridization was carried out with pPCY64, a chloroplast DNA probe originating from *Petunia hybrida* (De Haas et al. 1986), which was radioactively labeled by nick translation.

Screening for GUS activity

Callus of 39 putative fusion products (approximately 20 mg) was ground in an Eppendorf tube in 20 µl GUS-extraction buffer (Jefferson 1987) to which 1 µl of a 25 m*M* MUG (=4-methyl umbelliferyl glucuronide) solution was added. After incubation at 37 °C for 30 min the reaction was stopped by adding 20 µl 1 *M* Na_2CO_3. The tubes were centrifuged at maximum speed in an Eppendorf centrifuge for 5 min and subsequently placed in UV light, together with a negative and a positive control tube. The presence or absence of fluorescence was determined.

Isoenzyme analysis

To determine whether the green calli, obtained after fusion of albino ALRC protoplasts and leaf protoplasts of 7322-K1, expressed genes from both parents, isoenzyme analyses were performed for two enzymes: glutamate oxaloacetate transaminase (GOT) and malate dehydrogenase (MDH). Callus tissue (0.5 mg) was ground in 0.5 ml of ice cold buffer consisting of 0.1 *M* TRIS-HCl, 5% w/v Ficoll 400, 1% v/v β-mercaptoethanol and 0.05% Bromophenol Blue. The pH of the extraction buffer was 8.9 for GOT analysis and 7.0 for MDH analysis. After centrifugation in an Eppendorf centrifuge for 5 min at maximum speed, 20 µl of the supernatants was applied to a non-denaturing polyacrylamide gel, 6% for GOT, 8% for MDH.

Electrophoresis was carried out in a Desaphor VA (Desaga) separation system at 4 °C. The electophoresis buffer consisted of 0.02 *M* TRIS-Glycine, pH 8.75 for GOT or TRIS-Citric acid buffer, pH 7.0 for MDH. To test for GOT a voltage of 150 V was applied until the tracking dye band had migrated through the stacking gel (±30 min), then the voltage was increased to 180 V (62 mA) for 16 h. When MDH analysis was performed the starting voltage was 30 V, which after 30 min was increased to 190 V (350 mA) for 6 h. The gels were incubated in staining solutions prepared after Vallejos (1983) for 30 min (GOT) or several hours (MDH) at 37 °C in darkness.

Dot blot analysis using species-specific repetitive DNA probes

In order to determine the percentage of potato DNA in the fusion products dot blot analyses were performed. Total DNA from the fusion parents was isolated according to Rogers and Bendich (1988) after which the DNA was purified on a cesium chloride gradient. The DNA concentration was measured spectrophotometrically. Total DNA from 72 fusion calli was obtained using the method of Mettler (1987).

Twelve concentrations, ranging from 0 ng up to 275 ng, of ALRC or 7322 DNA, in addition to ±200 ng DNA from fusion calli, were applied to a Gene Screen Plus (GS^+) filter (Du Pont) using the HYBRI DOT 96-well filtration manifold from GIBCO/BRL, as follows. Whatman 3MM paper and GS^+ filter, soaked in 0.5 *N* NaOH, were placed in the manifold and clamped tightly. After the DNA solutions (50 µl) were pipetted in the wells, vacuum was applied. The wells were rinsed with 100 µl TE, pH 7.8. After another 5–10 min under vacuum the manifold was disassembled and the filter was placed on 3 MM paper that had been soaked in 0.5 *N* NaOH, for 5 min. Then the filter was air dried for 2 min, after which it was placed on 3 MM paper prewetted in 1 *M* TRIS-HCl (pH 7.5), for 2 min (2 times). The filter was again air dried for 2 min and subsequently placed on 3 MM paper soaked in 0.5 *M* TRIS-HCl (pH 7.5), 1.5 *M* NaCl, for 5 min. Finally the filter was air dried for 1 h at room temperature, wrapped in Saran-Wrap and kept at 4 °C until use.

Two identical filters were prepared for every analysis. One was probed with pTHG2, a tomato-specific repetitive DNA probe, kindly provided by Dr. P. Zabel, Department of Molecular Biology, Wageningen Agricultural University (Zabel et al. 1985). The insert of pTHG2 represents a moderately repetitive DNA fragment that is evenly dispersed on all tomato chromosomes. The other filter was probed with P5L, a potato-specific repetitive DNA probe, kindly provided by Dr. R. G. F. Visser, Department of Plant Breeding, Wageningen Agricultural University (Visser et al. 1988). On potato chromosomes hybridization with this fragment occurs predominantly in the telomeric and centromeric regions. All chromosomes of the *Solanum phureja* genome (a tuber-bearing *Solanum* species) can be labeled.

Probes were radioactively labeled using the Boehringer Mannheim Random Primed DNA Labeling Kit. Hybridization was performed in glass bottles using a HYBAID hybridization oven (Hybaid) at 65 °C for 16 h. The blots were rinsed in 2 × SSC, 1% SDS, 0.1% tetra-sodium pyrophosphate (Na-PPi) at 65 °C for 10 min (2 times), in 0.2 × SSC, 1% SDS, 0.1% Na-PPi at 65 °C for 30 min (2 times), in 0.1 × SSC, 1% SDS, 0.1% Na-PPi at 65 °C for 30 min (1 time) and finally in 0.1 × SSC at 65 °C for 30 min. Autoradiography was performed on Konica X-ray film. Dots were cut out of the filters and put in scintillation vials, 5 ml of the liquid scintillation cocktail Ultima Gold (Packard) was added and radioactivity per dot was measured in a Tri-Carb Liquid Scintillation Analyzer (Packard), 10 min per dot.

The analysis was performed in duplo for 36 of the 72 calli tested and in triplo for 23 calli.

Results and discussion

Protoplast fusion and callus culture

By using albino cell suspension protoplasts and green leaf mesophyll protoplasts as fusion parents it was possible to detect heterofusion products under a UV microscope. The cytoplasm of the albino cell suspension protoplasts showed a yellow autofluorescence, while the chloroplasts of the leaf mesophyll protoplasts showed a red autofluorescence. Twenty-four hours after fusion the cytoplasms of both protoplasts involved in the fusion were completely mixed, and in part of the fusion products the two nuclei had already fused. Two months after protoplast fusion the first green calli were visible. Irradiation did not seem to reduce the frequency of green calli (putative hybrids) that could be obtained in the different fusion experiments (Table 1).

Only white calli were obtained from ALRC protoplast cultures. In previous experiments neither irradiated

Table 1. Number of protoplasts used for fusion of ALRC and 7322-K1, number of green calli obtained and number of GUS positive and GUS negative calli in symmetric (0 Gy) or asymmetric (50, 500 Gy) fusion experiments

Irradiation dose of 7322-K1 plants	Number of protoplasts used for fusion	Number of green calli	Number of calli	
			GUS^+	GUS^-
0 Gy	6.1×10^6	28	13	0
50 Gy	5.6×10^6	32	17	3
500 Gy	8.0×10^6	120	6	0

7322-K1 protoplasts nor unirradiated protoplasts of this genotype ever yielded microcalli. Only white calli developed in mixtures of unfused ALRC and 7322-K1 protoplasts. Because of these results it was assumed that all green calli obtained after fusion were products of a fusion event between ALRC and 7322-K1 protoplasts.

Chloroplast DNA analysis

In order to confirm that the green calli obtained after fusion contained 7322-K1 chloroplasts, total DNA was isolated from 13 putative fusion calli (3 0 Gy calli; 6 50 Gy calli; 4 500 Gy calli) and from leaves of the fusion parents. Chloroplast DNA probe pPCY64 showed a polymorphism between HinfI-digested tomato and potato DNA (see Derks et al. 1990). When HinfI-digested DNA from the green calli was probed with pPCY64 all 13 calli contained the potato chloroplast DNA band, and none of them contained the tomato chloroplast DNA band (Fig. 1), indicating the presence of only potato chloroplasts in all of the calli tested.

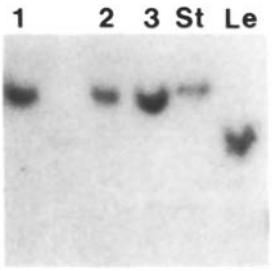

Fig. 1. Chloroplast DNA analysis of the putative fusion calli. Total DNA was isolated from *Lycopersicon esculentum* ALRC (*Le*), *Solanum tuberosum* 7322 (*St*) and from three putative fusion calli: *1* 50 Gy, nr 4; *2* 50 Gy, nr 13; *3* 500 Gy, nr 13. Approximately 4 μg DNA was digested with HinfI, the fragments were separated on a 1.2% agarose gel and subsequently Southern blotted. Hybridization was carried out with pPCY64, a chloroplast DNA probe (see Materials and methods)

GUS activity screening and isoenzyme analysis

To assay whether nuclear-encoded traits from 7322-K1 were still present in the green calli, a GUS activity test and isoenzyme analyses were performed. All 13 0 Gy calli, 17 out of 20 of the 50 Gy calli and all 6 500 Gy calli tested were positive for GUS activity (Table 1). This indicated that in most of the green calli a nuclear-encoded trait derived from 7322-K1 was expressed.

Isoenzyme analyses of the green calli are shown in Fig. 2. All 18 calli analysed for MDH isoenzymes showed a sum of the bands from both fusion parents (Fig. 2 A). In all 36 calli tested for the isoenzymes of GOT (a dimeric enzyme) not only parental bands were visible, but also a new hybrid band could be seen (Fig. 2 B). This new band confirms the hybrid nature of the green calli.

Quantitative analysis of the nuclear composition by dot blotting

All calli tested for MDH and GOT isoenzymes not only contained tomato nuclear DNA, but also potato genomic DNA, indicating that elimination of genetic material caused by the irradiation treatment was limited. To estimate the percentage of potato DNA in the nuclei of the fusion products species-specific repetitive DNA probes were used as a measure of the total amount of DNA from one species. The species specificity of probes pTHG2 and P5L was tested by Southern bot analysis (data not shown). Cross-hybridization of pTHG2 with potato DNA and of P5L with tomato DNA was negligible compared to the hybridization signal with homologous DNA.

Total DNA was isolated from the fusion parents and from 15 0 Gy calli, 24 50 Gy calli and 33 500 Gy calli. Several concentrations of parental DNA were applied to two identical dot blots to make a calibration plot of the radioactivity per dot in relation to the amount of DNA from one species (Fig. 4). Total DNA from the fusion products was applied to the same dot blots (Fig. 3). With the calibration plots for both species-specific probes the amount of tomato and potato DNA per dot could be estimated, and the percentage of nuclear DNA of the fusion products that originated from potato could be determined. Averages of the obtained data per fusion callus are shown in Fig. 5. The average difference between duplicate or triplicate determinations of the percentage potato DNA in the nuclei of individual fusion calli was 2.0%.

All fusion calli contained potato nuclear DNA. The large variation in percentage potato DNA in the group of symmetric fusion calli (Fig. 5) can be explained by the fact that the ALRC protoplasts were isolated from a suspension culture in which different ploidy levels were present and/or due to multiple fusion events. A moderate decrease in mean percentage potato DNA was detected in asymmetric hybrids (50 Gy and 500 Gy calli) as compared to symmetric hybrids (0 Gy calli). Interestingly, there seems to be no difference in mean percentage potato DNA between 50 Gy and 500 Gy calli.

These results show a remarkable feature: no true cybrids lacking potato nuclear DNA have been obtained. Since selection of fusion products was based exclusively on the correction of the cytoplasmically encoded albino phenotype of the tomato parent, transfer of functional potato chloroplasts to the tomato cells should presumably be sufficient to obtain green calli. However, even when the potato protoplasts had been heavily irradiated before fusion, a relatively large amount of potato nuclear DNA was retained in the hybrid calli.

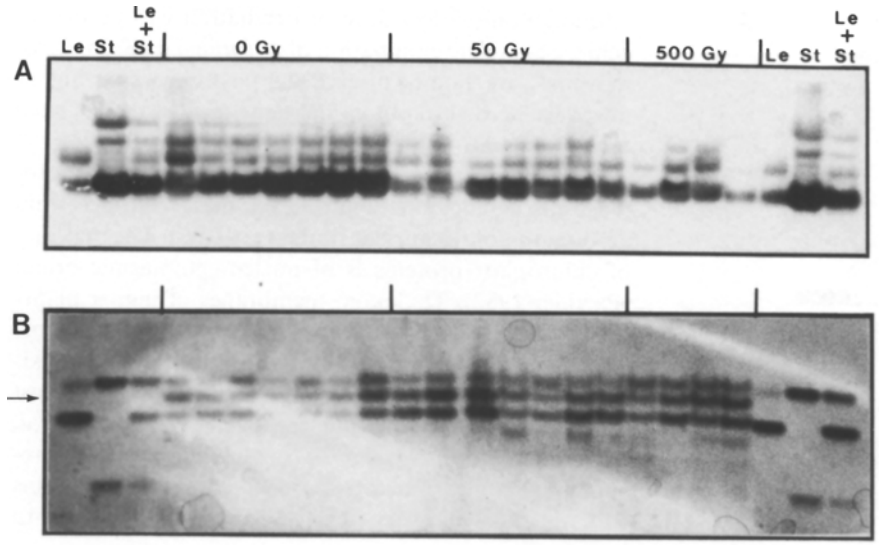

Fig. 2A, B. Isoenzyme analysis of the putative fusion calli. **A** Stained for MDH activity; **B** stained for GOT activity. *Le* Enzymes of *Lycopersicon esculentum* ALRC, *St* enzymes of *Solanum tuberosum* 7322-K1, *Le+St* mixture of enzymes of ALRC and 7322-K1, *0 Gy, 50 Gy, 500 Gy* enzymes of some green calli obtained after fusion of ALRC protoplasts with 0 Gy, 50 Gy or 500 Gy gamma-irradiated 7322-K1 protoplasts, respectively. *Arrow* in **B** indicates new intermediate-sized band present in all fusion calli

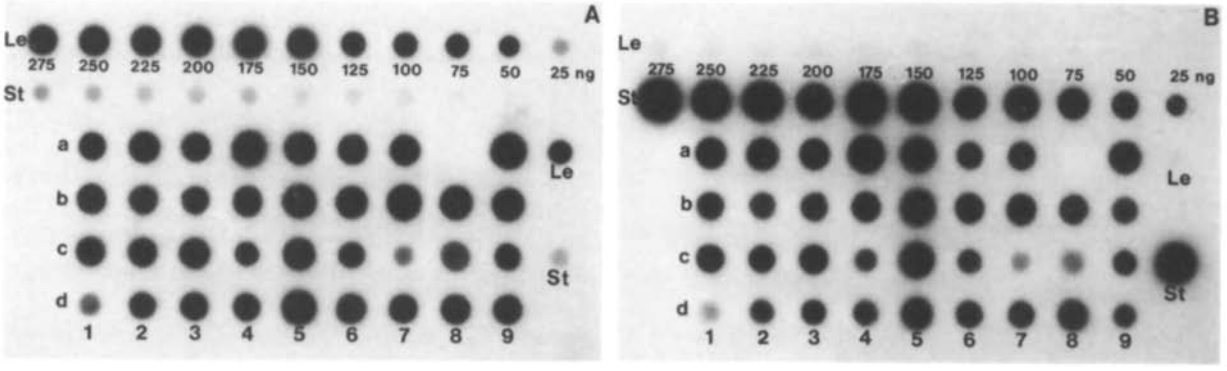

Fig. 3A, B. Dot blot analysis of fusion calli. Total DNA was isolated from the fusion parents *Lycopersicon esculentum* ALRC (*Le*) and *Solanum tuberosum* 7322 (*St*) and from green calli obtained after fusion of ALRC protoplasts with 0 Gy, 50 Gy or 500 Gy gamma-irradiated 7322-K1 protoplasts. Concentration series of ALRC DNA or 7322 DNA were applied to two identical dot blots besides ±200 ng DNA of 0 Gy (*a1–7*), 60 Gy (*a9*, *b1–9*, *c1–2*) and 500 Gy (*c3–9*, *d1–9*) fusion products. Blot **A** was probed with pTHG2, a tomato-specific repetitive DNA probe; blot **B** was hybridized with P5L, a potato-specific repetitive DNA probe

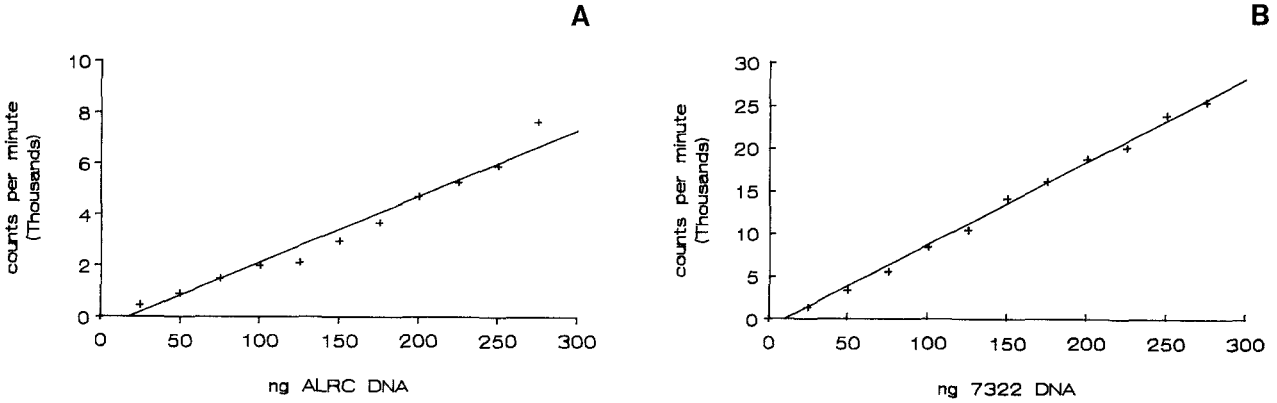

Fig. 4A, B. Calibration plots of the radioactivity per dot in relation to the amount of ALRC DNA (**A**) or 7322 DNA (**B**). Dots were cut out of the filters in Fig. 3, and radioactivity per dot was measured in a Liquid Scintillation Analyzer. Background radioactivity was subtracted from the obtained values

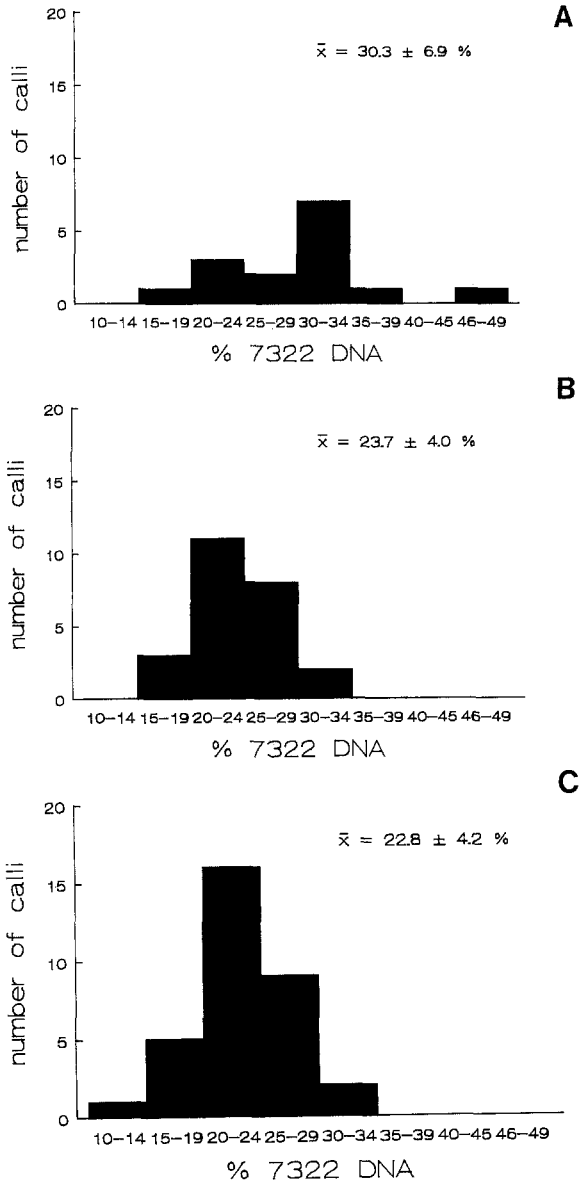

Fig. 5A–C. Mean percentage potato DNA in calli obtained after fusion of protoplasts of tomato genotype ALRC with protoplasts of 0 Gy (**A**), 50 Gy (**B**) or 500 Gy (**C**) gamma-irradiated potato 7322-K1

A possible explanation for the results presented here is that a dose of 500 Gy of gamma rays is not sufficient to cause total elimination of potato chromosomes from the fusion products. Menczel et al. (1982) fused gamma irradiated *N. tabacum* SR1 protoplasts (2n = 4x = 48) carrying a chloroplast DNA-encoded streptomycin resistance with *N. plumbaginifolia* protoplasts. Four doses of irradiation were used: 50, 120, 210 and 300 Gy. Similarly to the work described herein, selection of hybrids was based exclusively on a cytoplasmic trait: i.e. streptomycin resistance. However, following all four irradiation regimes, cybrids containing *N. plumbaginifolia* nuclear DNA and *N. tabacum* chloroplasts could be obtained. Although only a low dose of irradiation was needed to achieve complete elimination of *Nicotiana tabacum* chromosomes, we cannot discard the possibility that higher doses are needed to obtain the same result with *Solanum tuberosum*.

Alternatively, it is conceivable that for potato chloroplasts to remain functional in a tomato cell, a certain amount of potato nuclear traits is required. The majority of chloroplast proteins is of nucleo-cytoplasmic origin (Parthier 1982). Thylakoid membranes of higher plants are composed of approximately 45 unique protiens, most of which exist as four multiprotein complexes. Approximately half of the proteins are encoded by chloroplast DNA, the other half by nuclear DNA (Herrmann et al. 1985). Therefore, *Solanum tuberosum* chloroplasts presumably require nuclear-encoded chloroplast proteins from the same or a very closely related species in order to be functional (green).

However, it is somewhat surprising that the relatively closely related *Lycopersicon* species cannot replace these (in potato chloroplasts). The genera *Lycopersicon* and *Solanum* are members of the same tribe (Solaneae) in the family of Solanaceae, have nearly identical karyotypes and contain comparable monoploid amounts of nuclear DNA (0.7 pg). In addition to the homology of DNA sequences throughout the tomato and potato genomes, the linkage order of those sequences on the chromosomes is remarkably well conserved (Bonierbale et al. 1988). Analysis of restriction digests demonstrated also that, while the plastomes of potato and tomato are distinct, they appear to be closely related (Schiller et al. 1982).

Symmetric tomato-potato hybrids carrying chloroplasts from either species could be obtained (Schiller et al. 1982), presumably because the corresponding nuclear genome was always present. However, Derks et al. (in preparation) could not obtain true cybrids when the albino tomato ALRC was fused with *Solanum commersonii* or *S. etuberosum*. Similarly, Jain et al. (1988) could not isolate cybrids containing the tomato nucleus with atrazine-resistant chloroplasts derived from *S. nigrum*.

Genome-plastome incongruity has been reported in several other cases after protoplast fusion of different Solanaceous species. Examples are *Nicotiana/Solanum* (Thanh and Medgyesy 1989). *Atropa/Nicotiana* (Kushnir et al. 1991) and even some combinations within the genus *Solanum*, studied by Perl et al. (1990). An indication that within the genus *Lycopersicon* some chloroplast-nucleus combinations may not result in viable cybrids comes from the study of Bonnema et al. (1991), who fused 1,000 Gy gamma-irradiated *Lycopersicon pennellii* protoplasts with iodoacetamide-treated *L. esculentum* protoplasts. The chloroplast genotype of 65 regenerants was determined, and all contained the tomato plastome.

This genome-plastome incongruity, which has also been reported for some sexual hybrids (reviewed by Kirk

and Tilney-Bassett 1978), could be overcome in the *Nicotiana/Solanum* somatic hybrids by virtue of recombination between parental chloroplast DNA types (Thanh and Medgyesy 1989). However, chloroplast DNA recombination is presumably a very rare event in higher plants (Medgyesy 1990), and the present study provides further evidence on strong limitations in combining plastomes from one species with the nucleus from another, not very closely related species.

It can not be excluded that the limited elimination of nuclear DNA in asymmetric hybrids is also due to the fact that many "collaborating" nuclear genes of the donor species have to stay together in the hybrid to yield viable asymmetric somatic hybrid plants.

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