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The role of irradiation dose and DNA content of somatic hybrid calli in producing asymmetric plants between an interspecific tomato hybrid and eggplant

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Abstract Highly asymmetric somatic hybrid plants were obtained by PEG/DMSO fusion of gamma-irradiated mesophyll protoplasts of the kanamycin-resistant (KmR^+) interspecific hybrid *Lycopersicon esculentum* × *L. pennellii* (EP) with mesophyll protoplasts of *Solanum melongena* (eggplant, E). Elimination of the EP chromosomes was obtained by irradiating the donor genome with different doses of gamma rays (100, 250, 500, 750 and 1000 Gy). The selection of somatic hybrid calli was based on kanamycin resistance; EP and E protoplasts did not divide due to the irradiation treatment and sensitivity to kanamycin, respectively. KmR^+ calli were recovered following all irradiation doses of donor EP protoplasts. The hybrid nature of the recovered calli was confirmed by PCR amplification of the *NptII* gene, RAPD patterns and Southern hybridizations using potato ribosomal DNA and pTHG2 probes. Ploidy levels of calli confirmed as hybrid were further analyzed by flow cytometry. Such analyses revealed that the vast majority of hybrid calli that did not regenerate shoots were 5–9n polyploids. The three asymmetric somatic hybrid plants obtained were regenerated only from callus with a ploidy level close to 4n, and such calli occurred only when the donor EP had been exposed to 100 Gy. The amount of DNA in somatic hybrid calli, from 100-Gy exposure, was found by dot blot hybridization with the species-specific probe, pTHG2, to be equivalent with 3.1–25.8% of the tomato genome. Thus, DNA contained in 3.8–13.2 average-size tomato chromosomes was present in these hybrid calli. The asymmetric somatic hybrid plants had the eggplant morphology and were regenerated from one hybrid callus that contained an amount of tomato DNA equivalent to 6.29 average-size tomato chromosomes.

Key words Eggplant · Tomato · Asymmetric somatic hybrids · Flow cytometry · Dot blot hybridization

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Introduction

Biotechnologies for plant improvement are of interest because they circumvent the limitations of traditional crop breeding. For example, somatic hybridization overcomes sexual incompatibility between species, and many somatic hybrids between combinations of plant species have been reported (Gleba and Sytnik 1984). Eggplant (*Solanum melongena*) is an important vegetable crop in Asian, tropical and European countries, and efforts for improving it by the introgression of germ plasm from selected *Solanaceae* species has included somatic hybridization with wild *Solanum* species: *S. sisymbriifolium* (Gleddie et al. 1986), *S. khasianum* (Sihachakr et al. 1988), *S. nigrum* (Guri and Sink 1988a), *S. torvum* (Guri and Sink 1988b) and *S. aethiopicum* (Daunay et al. 1993).

The irradiation of donor protoplasts is now commonly employed to direct chromosome elimination for the creation of asymmetric hybrids. Irradiation dose-dependent elimination of donor chromosomes in interspecific somatic hybrids has been reported by a number of authors (Melzer and O'Connell 1992; Kovtun et al. 1993; Trick et al. 1994). Conversely, in fusion experiments with taxonomically remote species no correlation between the level of irradiation and the extent of asymmetry in somatic hybrids has been observed (Dudits et al. 1987; Wolters et al. 1991). However, irradiation has been observed to direct the elimination of the donor genome (Gleba et al. 1988).

The genus *Lycopersicon* is a resource of valuable agronomic traits (Daunay et al. 1991), and consequently it would be expedient to introduce some of these traits into the gene pool of *Solanum melongena* via somatic hybridization. Eggplant (+) *L. esculentum* × *L. pennellii* non-regenerable hybrid calli have been previously described by Guri et al. (1991). In the same fusion combination [*S. melongena* (+) *L. esculentum* × *L. pennellii*] Liu et al. (1995) reported the regeneration of somatic hybrid plants with a leave shape similar to that of tomato and branching that resembled that of eggplant. However, these authors did not carry out molecular analysis of the somatic hybrids. It

would be interesting to monitor donor chromosome flow to the recipient eggplant in order to obtain asymmetric somatic hybrids. As reported herein such an analysis was conducted in the production of highly asymmetric somatic hybrid plants following fusion of γ-irradiated donor protoplasts of *L. esculentum* × *L. pennellii* with recipient protoplasts of *S. melongena*.

Materials and methods

Plant materials

Seeds of *S. melongena* L. (line 410, 2n=2x=24) were provided by Dr. S. Izhar, ARO, Israel and those of cv 'Imperial Black Beauty' were obtained from Stokes Seeds, Buffalo, N.Y. Seeds of *L. esculentum* cv 'VF36' and *L. pennellii* LA716 were provided by Dr. C. M. Rick, Tomato Genetics Resource Center, University of California. Kanamycin-resistant plant A54 of the sexual hybrid between *Lycopersicon esculentum* Mill. (cv 'VF36', 2n=2x=24) × *L. pennellii* L. (cv 'LA716', 2n=2x=24), herein EP, was provided by Dr. R. Jorgensen, DNAP, Oakland, Calif. EP has a T-DNA insert mapped to *L. esculentum* chromosome 12 (Chyi et al. 1986).

Protoplast isolation, irradiation and fusion

Protoplasts were isolated from the leaves of 3- to 4-week-old seedlings of *S. melongena* according to Guri and Izhar (1984). Protoplasts of EP, which were obtained from plants grown in vitro under standard conditions, were isolated as described by Tan et al. (1987) with some modifications. Plants 3–4 weeks old were transferred to the dark for 24–48 h and then pretreated at 4°C for 4–12 h in the dark. The concentration of the enzymes Cellulysin (Calbiochem) and Macerozyme R10 (Serva) was increased to 0.7% and 0.2%, respectively.

Prior to fusion protoplasts of EP were irradiated with 100, 250, 500, 750 or 1000 Gy of [^{60}Co]gamma rays (9.6 Gy/min) at 4°–10°C. After irradiation, the protoplasts were washed once with W5 solution (Medgyesy et al. 1980) and mixed with those of *S. melongena* in a 1:1 or 1:3 ratio to give a final density of 1×10^6 protoplasts per milliliter. The PEG-DMSO fusion procedure (30% PEG, mol. wt. 4000) was conducted according to Menczel et al. (1981).

Protoplast culture and plant regeneration

After fusion, protoplasts were cultured in the dark at 28°C for 2–3 days in Kao medium (1977) supplemented with MS Fe-EDTA and 0.5 g/l MES. On the third day of culture cells were embedded in 1.4% alginate and cultured in 3 ml of medium (60×15 mm petri dishes) in the dark at 28°C. Two weeks later the alginate sections were transferred to 100×15 mm petri dishes, and 10 ml of fresh medium (Kao 1977) plus kanamycin at 25 mg/l rate were added. Four weeks after fusion the alginate sections were dissolved in 0.04 *M* sodium citrate, and the released micro-calli were cultured with 10–15 ml of C medium (Shepard et al. 1982) containing 25 mg/l kanamycin and exposed to light (200 $\mu Em^{-2}\ s^{-1}$). Two-three weeks later green calli (1–2 mm in diameter) were retrieved individually and placed on MS (Murashige and Skoog 1962) medium supplemented with 2% sucrose, 2 mg/l zeatin, 0.1 mg/l IAA, 25 mg/l kanamycin and 0.6% Noble agar (Difco) (Guri and Sink 1988b). When leaf-like primordia appeared the calli were subcultured on MS medium as above, but supplemented with 100 mg/l adenine sulfate, 20 ml/l coconut water, 0.01 mg/l biotin, 0.5 mg/l folic acid and 0.05 mg/l GA_3. Regenerated shoots were transferred to solidified MS supplemented with 0.2 mg/l IAA for rooting. After 2–3 weeks rooted plants were transferred to the soil and placed in a greenhouse.

DNA extraction

DNA was isolated from in vitro-growing plants and calli according to Doyle and Doyle (1990). DNA concentrations were measured spectrophotometrically.

PCR (polymerase chain reaction) amplification of the *NptII* gene and RAPD analysis

PCR amplification of a 255-bp *NptII* gene fragment was carried out with primers 5′>CGCAGGTTCTCCGGCCGCTTGGGTGG<3′and 5′>AGCAGCCAGTCCCTTCCCGCTTCAG<3′. These primers enclose a 255-bp fragment of the *NptII* gene (Beck et al. 1982). Amplifications were done on a Perkin-Elmer Cetus Thermal Cycler model 480 or 9600 with the program described by Lipp Joao and Brown (1993).

For RAPD (random amplified polymorphic DNA) amplification oligonucleotide primers (45) randomly chosen from different (Operon Technologies) kits were used: OPA-06, -09; OPB-15; OPC-03; OPD-13; OPE-02, OPE-18; OPH-01, 20; OPI-01 to 20; OPJ-01 to 03; OPK-01 to 03, -20; OPL-01, -06, -10 to 16. The RAPD reactions were each carried out in 25-μl volumes, each containing 25 ng of target DNA; 1.25 μm of the primer; 200 μ*M* each of dATP, dCTP, dGTP, dTTP; 25 m*M* $MgCl_2$ and 2 units of Stoffel Fragment polymerase (Perkin Elmer) or 1.3 units of *Taq* polymerase (Boehringer Mannheim) in the corresponding buffer. Amplifications were performed on a Perkin Elmer Cetus 9600 thermal cycler as described by Williams et al. (1990).

Amplified products were loaded into 1.2% agarose gels and electrophoresed in TBE buffer for 6 h at 120 V. DNA was visualized with ethidium bromide. Images were scanned using a Gel Print 2000i system (BioPhotonics) with Mitsubishi video copy processor P40 U.

Southern and dot blot analysis

For Southern analysis approximately 5 μg genomic DNA was digested with 40 U of restriction enzyme (*Eco*RI, *Eco*RV, *Hin*dIII) for 16 h. Fragments were separated by electrophoresis for 16 h on 0.9% agarose gels in TAE buffer. DNA was transferred to Hybond-N+ (Amersham) nylon membranes, and Southern hybridizations were performed as described by Sambrook et al. (1989). The two probes used for hybridization were a potato ribosomal DNA probe supplied by Dr. D. Douches, Michigan State University, and a tomato species-specific repetitive DNA probe, pTHG2 (Zabel et al. 1985), provided by Dr. P. Zabel, Wageningen Agricultural University, The Netherlands.

For dot blot analysis, serial dilutions (0–275 ng/dot) of genomic DNA from *L. esculentum* cv 'VF36', *L. pennellii* 'LA716', *L. esculentum* × *L. pennellii* (EP) and *S. melongena*, along with 275 ng (in triplicate) of DNA from each hybrid calli, were applied on Hybond-N (Amersham) nylon membranes according to the manufacturer's recommendations using the Schleicher and Schuell Minifold Apparatus (Keene, USA). The amount of tomato DNA in hybrid calli was quantified with probe pTHG2.

Probes were labeled with [^{32}P] using the Random Primer DNA Kit (Gibco, BRL). Hybridization was visualized and quantified by using the Molecular Dynamics PhosphorImager (Sunnyvale, USA) with a Zeos computer system running Molecular Dynamics ImageQuant (v. 33) software. The filters were exposed to Kodak X-OMAT film for documentation.

Flow cytometric analysis

In vitro-growing parental plants and somatic hybrid calli confirmed by previous assays were used for nuclei isolation. Preparation of the nuclei for flow cytometric analysis and calculation of plant nuclear DNA were performed as described by Arumuganathan and Earle (1991b). Nuclei from chicken red blood cells (CRBC) (Colorado Serum) or mouse thymocytes (Thy) were used as internal reference stan-

Table 1 Recovery of calli and analyses conducted following somatic hybridization between different irradiation levels on donor EP protoplasts fused with eggplant

Irradiation dose (Gy)	Fusion ratio EP:E	Surviving calli (no.)	KmR^+ calli (no.)	Number of calli analyzed		
				PCR	RAPD	Flow cytometry
100	1:1	5.2×10^3	123	11	11	5
250	1:2	1.4×10^4	9	5	5	2
500	1:3	3.7×10^3	6	5	3	–
750	1:3	1.3×10^4	53	5	5	4
1000	1:3	1.2×10^4	11	4	4	4

– Not determined

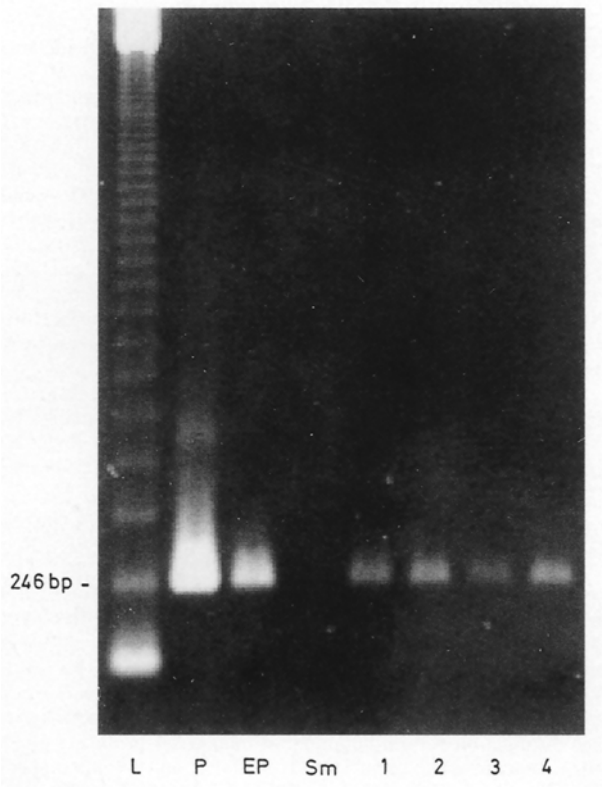

Fig. 1 PCR amplification of the *NptII* gene. *P* Plasmid DNA, *EP L. esculentum* × *L. pennellii*, *Sm Solanum melongena*, *lanes 1–4* somatic hybrid calli (100 Gy) H11, H14, H17, H18, respectively, *L* a 123-bp marker ladder

dards by which to compute the DNA content of 2C somatic hybrid calli and parental plant nuclei, respectively. CRBC were also used to calculate the DNA content of ethanol-fixed Thy (Fig. 4A). The value of 2.33 pg/2C (Galbraith et al. 1983) was used for the absolute DNA content of CRBC. The fluorescence of nuclear DNA was measured using a Becton Dickinson FACS Vantage flow cytometer with Consort 32 (HP 340) computer system running LYSIS II acquisition and analysis software. Data were analyzed using Multiplus Av software (Phoenix Flow Systems). Background fit was used to reduce DNA fluorescent noise from nuclear fragments (Fig. 4D).

Results

Protoplast culture and plant regeneration

Somatic hybrid calli were recovered following PEG/DMSO fusion using all γ-irradiation levels (100, 250, 500, 750 and 1000 Gy) on donor protoplasts of EP (+) eggplant. Our experiments confirmed earlier results from this laboratory (Guri et al. 1991; Liu et al. 1995) in which kanamycin at 25 mg/l completely inhibited the growth of *S. melongena* explants and calli, and simultaneously had no adverse influence on the growth of *L. esculentum* × *L. pennellii* (EP). Variable levels of γ-rays on EP protoplasts revealed that doses of 100 Gy and higher prevented any colony formation.

The initial plating efficiency of the eggplant protoplasts, when the plating protocol of Guri and Izhar (1984) was used, was 20–25%. However, only 900–1000 micro-calli (0.1–1 mm in diameter) were recovered per control fusion (EP and eggplant protoplasts mixed in a 1:1 ratio at 5×10^6 protoplast/ml). Consequently, several experiments were carried out to improve the plating efficiency of the fusion products using different culture media (Kao 1977; Murashige and Skoog 1962) and embedding protoplasts in 1.4% alginate. Optimum results were achieved with modified KM culture medium (see Materials and methods) combined with alginate culture, which yielded up to 1.4×10^4 cell colonies per fusion. Subsequently, in fusions with γ-irradiated donor protoplasts, a total of 208 KmR^+ calli were selected among which, 123, 9, 6, 53 and 17 were obtained from 100, 250, 500, 750 and 1000 Gy, respectively (Table 1). The vast majority of calli produced in all irradiated donor-recipient fusion combinations turned pale white and died within 15 months post-fusion on selective medium.

PCR, RAPD and Southern analysis

Out of the 208 kanamycin-resistant calli recovered from the fusion of irradiated protoplasts with eggplant protoplasts 30 were analyzed (Table 1). Each of these calli had the 255-bp *NptII* fragment (Fig. 1). For RAPD analysis, the 8 primers that produced the most distinct banding profiles (OPH-01, -20; OPI-01, -02, -07, -10; OPK-01, -20) were used. Among the 11 calli recovered in the 100-Gy donor EP (+) eggplant protoplast fusions that were analyzed, 5 had RAPD patterns similar to that of tomato although 2 of them, H4 and H16, contained additional polymorphic bands that were not present in either fusion parent. The other 6 calli (H11, H14, H17, H18, H20 and H21) were confirmed as hybrids although they had banding patterns mostly derived from eggplant (Fig. 2). Banding profiles of kanamycin-resistant calli selected and analyzed from

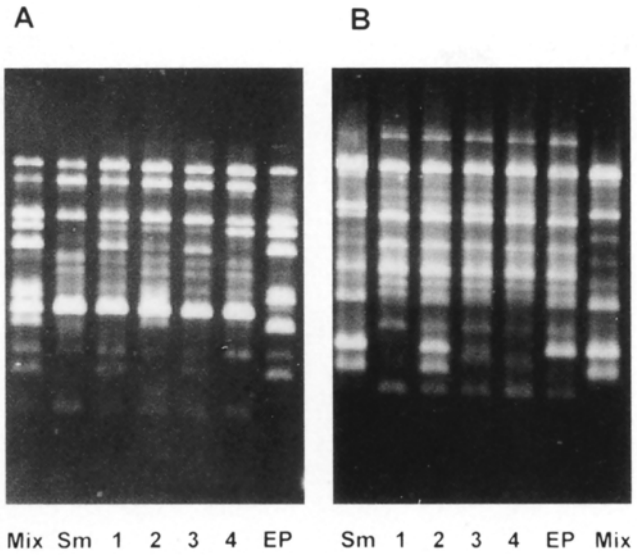

Fig. 2A,B Amplification of genomic DNA by decamer primers H-01 (**A**) and K-01 (**B**). *Mix* Mixture of parental DNA, *Sm S. melongena*, *lanes 1–4* somatic hybrid calli (100 Gy) H11, H14, H17, H18, respectively, *EP L. esculentum* × *L. pennelii*

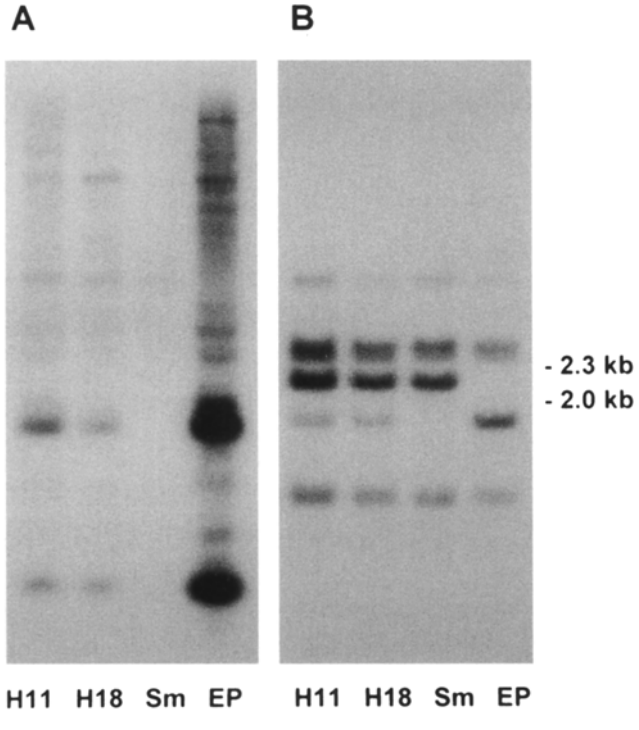

Fig. 3A,B Southern blot hybridization of genomic DNA from *L. esculentum* × *L. pennelii* (*EP*), *S. melongena* (*Sm*) and somatic hybrid calli *H11*, *H18*. DNA was digested with *Eco*RI (**A**) and *Hin*dIII (**B**) and hybridized with tomato-specific probe pTHG2 (**A**) and potato rDNA probe (**B**)

higher doses of irradiation, 5, 3, 5 and 4 from 250, 500, 750 and 1000 Gy, respectively, although predominately eggplant also had EP bands (data not shown).

Two calli, H11 and H18 (100 Gy), confirmed as hybrids by RAPD analysis, were further analyzed for the presence of tomato DNA by Southern hybridization. Such hybridizations with the tomato species-specific repeat pTHG2 and the potato rDNA probe revealed the presence of limited amounts of EP DNA (Fig. 3A,B). Also, 5 other calli obtained from 100 Gy, mentioned previously as having only tomato RAPD pattern(s), had tomato hybridization profiles.

Flow cytometric analysis

The nuclear DNA content of eggplant and tomato (Arumuganathan and Earle 1991a) is close to that of chicken red blood cells (Galbraith et al. 1983). Thus, CRBC were used as a standard for mouse thymocytes (Fig. 4A) and somatic hybrid calli (Fig. 4D). Conversely, Thy were used as the internal standard to determine the nuclear DNA content of the parental lines involved in somatic hybridization (Fig. 4B,C). The nuclear DNA content of ethanol-fixed Thy was found to be 6.137±0.262 pg/2C. Therefore, the nuclear DNA of *S. melongena* – 2.479±0.058 pg/2C nuclei – and EP – 2.017±0.189 pg/2C nuclei – could be calculated. The nuclear DNA content of hybrid calli from donor EP subjected to different doses of γ-irradiation ranged from 5.498 pg/2C (H18) to 20.163 pg/2C (H11). All of the calli tested (Table 2) were polyploids (Fig. 4E); for instance, H18 – 4.4n, H11 – 16.3n (100 Gy); H251 – 9.2n, H252 – 9.6n (250 Gy); H754 – 6.7n, H751 – 9.2n (750 Gy); H1003 – 8.7n, H1002 – 9.2n (1000 Gy). Hybrid plants regenerated only from callus H18 with 5.49 ng/2C, a DNA content close to the 4n of eggplant, and such calli only occurred when the donor was irradiated with 100 Gy. Analysis of hybrid plant H18-1 revealed that it has a 2C value of 5.47 pg.

Dot blot analysis

The amount of EP DNA in 7 calli and two hybrid plants, all obtained from the 100-Gy exposure, was determined by dot blot analysis (Fig. 5B). The relative amount of *L. esculentum* repetitive sequences in genomes of *L. pennellii*, *L. esculentum* × *L. pennellii* (EP) and eggplant was calculated first in order to be able to score the amount of EP genome in each hybrid calli. Tomato species-specific 452-bp repeat pTHG2 is randomly located in all chromosomes of *L. esculentum* (Zabel et al. 1985); thus, the value of hybridization of pTHG2 to *L. esculentum* DNA was used as the standard at 100%. The value of hybridization of probe pTHG2 to *L. pennellii* and EP DNAs relative to *L. esculentum* DNA was found to be 100%. The hybridization signal of the pTHG2 probe to *S. melongena* DNA was negligible (Fig. 5A). The mean of the background was subtracted from the values obtained. The calibration plot (Fig. 6) shows the linearity of hybridization of pTHG2 in a concentration series of DNA from *L. esculentum* (Le), *L. pennellii* (Lp) and EP. Thereafter, the percentage of tomato repeats in somatic hybrid calli was determined by a comparison of the degree of hybridization of pTHG2 to

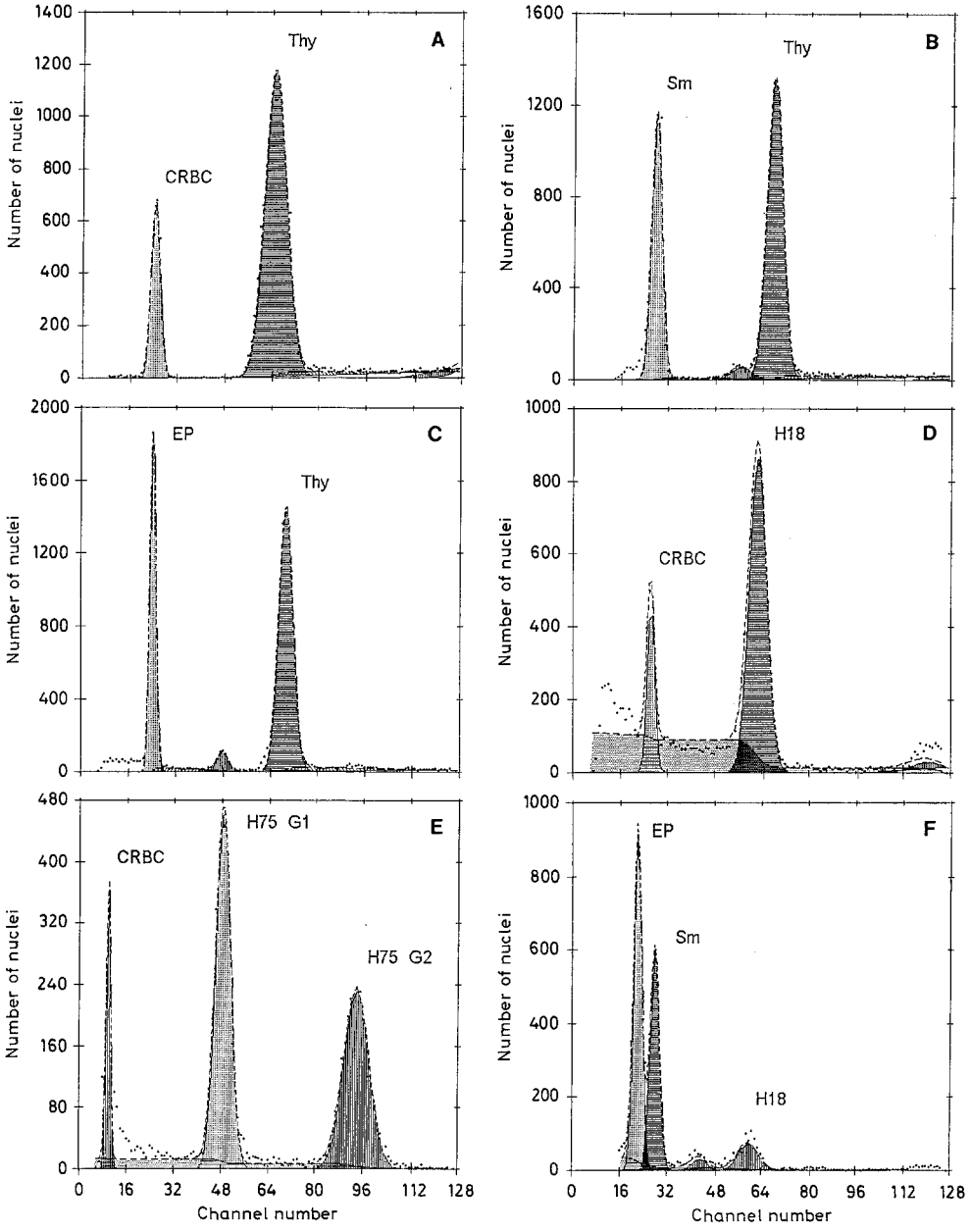

Fig. 4A–F Flow cytometry histograms of nuclei. **A** Chicken red blood cells (*CRBC*) and mouse thymocytes (*Thy*), **B** *S. melongena* (2n=2x=24) (*Sm*) and *Thy*, **C** *L. esculentum* × *L. pennellii* (2n=2x=24) (*EP*) and *Thy*, **D** CRBC and a somatic hybrid callus *H18* (100 Gy), **E** CRBC and somatic hybrid callus *H75* (750 Gy), **F** parental – *EP*, *Sm* and somatic hybrid callus *H18*

DNA from the hybrid calli with that hybridizing to EP (Fig. 5B). Calculations of EP chromosomes in somatic hybrid calli are based on the assumption that a symmetrical hybrid with all of the chromosomes of eggplant (E) and EP should have a DNA content of 4.496 pg and a corresponding dot blot value of about 44.862% of that obtained for an equal amount of EP genome. Thus, 1 EP chromosome should produce an average dot blot value of 1.869%. Hence, the percentage of DNA and consequently the number of EP chromosomes in hybrid calli was calculated and found to be 8.0 for H11, 6.2 for H14, 3.8 for H17, 6.29 for H18, and 13.2 for H20. Somatic hybrid plants H18-1 and H18-2 contained 4.77 and 5.14 EP chromosomes, respectively, and were regenerated from calli H18 with 6.29 EP chromosomes. Calli H4 and H16, from 100 Gy, were confirmed as being tomato by the presence of multiple copies of tomato DNA that corresponded to 118.9 and 107.2 EP chromosomes, respectively.

Only 1 callus, H18, only when the donor was irradiated at 100 Gy and after 12 months in culture, regenerated multiple shoots, among which three shoots, taken from independent sites, were recovered, rooted and transferred to the greenhouse. Morphologically, the putative somatic hybrids resembled eggplant but grew slower relative to the fusion parents. Further attempts to induce shoot regeneration from somatic hybrid calli from all irradiated donor-recipient fusion combinations were unsuccessful over a period of 2 years.

Table 2 Nuclear DNA content of somatic hybrid calli selected after somatic hybridization between different irradiation levels on donor EP protoplasts fused with eggplant

Irradiation dose (Gy)	Genotype	Nuclear DNA content 2C/pg
	Plants	
	S. *melongena*	2.479 ± 0.058
	EP	2.017 ± 0.189
	Calli	
100	H11	20.163
	H14	12.719
	H17	5.688
	H18[a]	5.498
250	H251	11.371
	H252	11.903
750	H751	11.415
	H752	10.805
	H753	11.225
	H754	8.244
1000	H1001	11.447
	H1002	11.346
	H1003	10.814
	H1004	11.143

[a] H18 regenerated hybrid plants H18-1 to -3

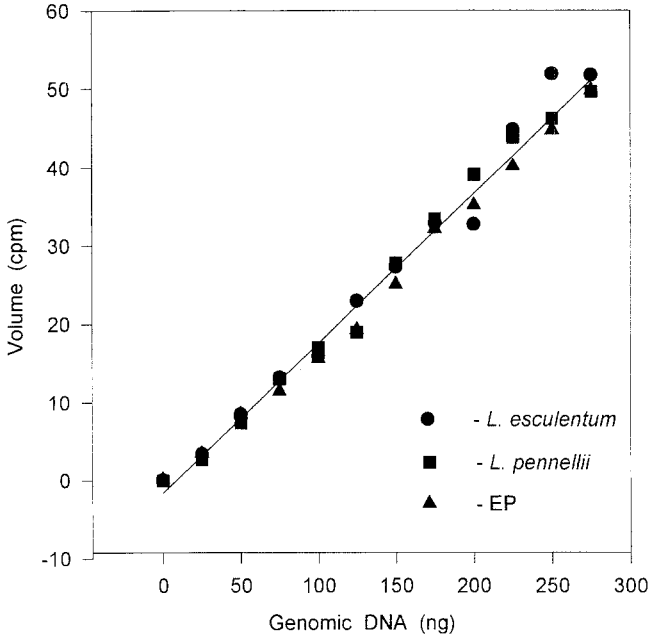

Fig. 6 Calibration plot of the radioactivity per dot in relation to the amount (ng) of genomic DNAs from *L. esculentum*, *L. pennellii* and *L. esculentum* × *L. pennellii* (*EP*)

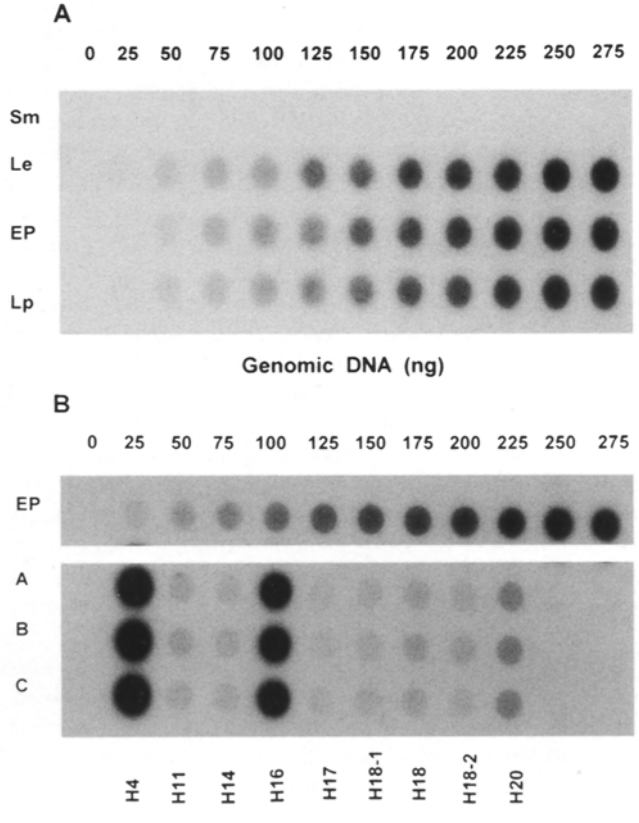

Fig. 5A,B Dot blot hybridization of genomic DNAs with tomato species-specific probe pTHG2. **A** *Sm S. melongena*, *Le L. esculentum*, *Lp L. pennellii*, *EP L. esculentum* × *L. pennellii*. **B** *EP L. esculentum*×*L. pennellii* and hybrid calli and plants obtained from 100-Gy experiment. DNA from somatic hybrids was loaded in triplicate – *A*, *B*, *C*

Discussion

The synthesis and recovery of intergeneric asymmetric somatic hybrid calli and plants of EP (+) E by the application of a wide range of γ-irradiation doses on donor EP was carried out. Success in such somatic fusion experiments depends on the efficiency of cell growth and the regeneration methods. In an earlier study of the same fusion partners used herein, only about 2×10^3 calli resulted from which two hybrid lines were selected (Guri et al. 1991). In the present study, the use of alginate culture and modification of the Kao (1977) medium yielded up to about 1.4×10^4 microcalli per fusion. From these microcalli, several hundred KmR^+ calli were selected, some of which were analyzed. The recovery of some calli having only tomato DNA in fusion experiments when the donor was irradiated with 100 Gy may be explained by the multiple self-fusions of donor protoplasts and complementation of irradiation damage resulting in the growth of cells with hexa- and higher ploidy levels.

The nuclear DNA content of hybrid calli resulting from all doses of γ-irradiation was similar and did not correlate with the dose applied. The majority of these somatic hybrid calli analyzed were 5–9n polyploids. However, somatic hybrid plants with eggplant morphology regenerated only from 1 callus that had a ploidy level close to 4n, and such calli occurred only in the 100-Gy donor EP (+) eggplant protoplast fusions. This morphogenic callus (H18) had 5.49 ng/2C which is, in fact, the 4n of eggplant and the amount of DNA equivalent to about 6.29 EP chromosomes.

It has been shown that species-specific repetitive DNA probes can be used in dot blots to measure the amount of donor DNA in asymmetric hybrids (Imamura et al. 1987). An analysis of *Nicotiana* asymmetric somatic hybrids by dot blot and chromosome counts (Piastuch and Bates 1990; Kovtun et al. 1993) revealed that the dot blot value was correlated with the number of chromosomes in the hybrids. Furthermore, both Daunay et al. (1993), in a study of somatic hybrids of eggplant, and Schoenmakers et al. (1993), in describing hybrids between tomato and potato, reported a correlation between chromosome counts and flow cytometry data. The correlation between flow cytometry and dot blot data established in this study enabled us to estimate the genome composition of selected EP (+) E calli. Flow cytometry data for the callus from which plants were regenerated, line H18, revealed that it had a DNA content of 5.49 pg, which is 1.22-fold larger than expected for the symmetric hybrid (4.496 pg), and approximately twofold larger than eggplant DNA content (E 4C=4.958 pg). Thus, with respect to the morphology of regenerated plants and the phenomenon of polyploidization of the recipient genome, which frequently occurs in "gamma" hybrids (Famelaer et al. 1989), hybrid H18 is an asymmetric callus with the 4C of eggplant and a low amount of EP genome. By our calculation, H18 has 4.958 pg of eggplant DNA and 0.532 pg of tomato DNA, which corresponds to the amount of DNA in 48 eggplant and 6.33 EP chromosomes, respectively. Thus, the genome of H18 contains approximately 9.69% EP genome.

The theoretical dot blot value for this progenitor callus would be 11.83% tomato and, in fact, the dot blot analysis value was 11.768%. The 11.768% corresponds to 6.29 EP chromosomes, which is close to the theoretical number of 6.33. These results indicate that flow cytometry analysis in conjunction with dot blot hybridization allows determination of both the ploidy level of hybrids and the amount of donor and recipient DNA present. Thus, the number of donor and recipient chromosomes in asymmetric hybrids may be calculated. This procedure is particularly valuable when chromosome counting by cytology is difficult and/or cannot be applied when chromosome morphology is similar as in the case of tomato and eggplant. Discrepancies between the flow cytometry and dot blot data in our experiments are in the range of those found in other studies (Piastuch and Bates 1990; Kovtun et al. 1993). They might also be related either to experimental errors of dot blot values and/or flow cytometry, or to an undetected non-random distribution of the repetitive sequences in the plant genome and/or the average DNA content of the chromosomes used for calculations.

Flow cytometric analysis of the parental species and asymmetric somatic hybrids, on the other hand, permitted the monitoring of chromosome elimination in asymmetric hybrid calli between phylogenetically remote species. Based on this analysis we suggest that in fusion experiments with phylogenetically remote species irradiation of the donor protoplasts in fact directs chromosome elimination, while the degree of elimination may depend on the size of the donor and recipient genomes, for example the ratio (donor:recipient) between DNA content of species involved in the fusion. For instance, Wolters et al. (1991) reported a limited degree of donor DNA elimination in somatic hybrid calli obtained after γ-irradiated protoplasts of *Solanum tuberosum* were fused with *L. esculentum*. These two species are characterized by a donor:recipient DNA ratio of 1.8:1 [DNA contents of *S. tuberosum* and *L. esculentum* are 3.31–3.86 (2n=4x) and 1.88–2.07 pg, respectively (Arumuganathan and Earle 1991a)]. In our fusion experiments between *L. esculentum* × *L. pennellii* (+) *S. melongena* with a donor:recipient DNA ratio of 1:1.22 (DNA contents are 2.017 and 2.479 pg, respectively) only 1 callus with 6.29 donor chromosomes resulted in the regeneration of asymmetric hybrid plants. Conversely, in fusion experiments with species characterized with a higher DNA ratio for the recipient – for instance, *Daucus carota* (donor) and *Nicotiana tabacum* (recipient) have a DNA ratio of 1:9.4 [DNA contents of *D. carota* and *N. tabacum* are 0.98 and 8.75–9.63 pg, respectively (Arumuganathan and Earle 1991a)] – Dudits et al. (1987) reported the regeneration of fertile somatic hybrid plants possessing only 1 chromosome of the donor. The spontaneous extensive chromosome elimination in intergeneric somatic hybrid plants between *Atropa belladonna* (donor) and *N. tabacum* (recipient), which were obtained without any pretreatment of the donor genome (Babiychuk et al. 1992), may also be explained by this theory. The fact is that when irradiation is used, an increase in the phylogenetic distance between the fusion partners does not necessarily promote the loss of donor chromosomes (McCabe et al. 1993). This relationship is in good agreement with our hypothesis, because the species-specific size of the nuclear genome does not depend on phylogenetic distance between species. Certainly, our hypothesis has to be further tested by somatic fusion experiments involving species with different size genomes.

Polyploidization of the recipient genome in somatic hybrids, which has been frequently observed (Gleba et al. 1988; Wijbrandi et al. 1990; McCabe et al. 1993) and was common in our experiments, could cause the low shoot regeneration frequency of hybrids, especially when three genomes of taxonomically remote species are mixed. Although regenerated plants were characterized by the difficulty in the rooting of shoots this cannot be taken directly as an indication of somatic incompatibility operating on the somatic hybrids since our preliminary study of *S. melongena* revealed some difficulty in the subculture of micro-cuttings in vitro due to a lack of rooting.

The asymmetric plants with genomes of *L. esculentum* × *L. pennellii* (+) *S. melongena* are being further analyzed by molecular and cytological means to determine the specific composition of donor chromosomes, organelles and possible chromosome recombination.

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