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UV irradiation as a tool for obtaining asymmetric somatic hybrids between *Nicotiana plumbaginifolia* and *Lycopersicon esculentum*

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Abstract UV-irradiated kanamycin-resistant *Lycopersicon esculentum* leaf protoplasts were fused with wild-type *Nicotiana plumbaginifolia* leaf protoplasts. Hybrid calli were recovered after selection in kanamycin-containing medium and subsequently regenerated. Cytological analysis of these regenerants showed that several (2–4) tomato chromosomes, or chromosome fragments, were present in addition to a polyploid *Nicotiana* genome complement. All lines tested had neomycin phosphotransferase (NPTII) activity and the presence of the kanamycin gene was shown by Southern blotting. In two cases a different hybridization profile for the kanamycin gene, compared to the tomato donor partner, was observed, suggesting the occurence of intergenomic recombination events. The hybrid nature of the regenerants was further confirmed by Southern-blotting experiments using either a ribosomal DNA sequence or a tomato-specific repeat as probes. The hybrids were partially fertile and some progeny could be obtained. Our results demonstrate that UV irradiation is a valuable alternative for asymmetric cell-hybridization experiments.

Key words Asymmetric cell hybridization · UV irradiation · Chromosome elimination · *Nicotiana* · *Lycopersicon*

Introduction

Somatic cell hybridization provides the possibility of circumventing barriers to sexual reproduction and allows for the improvement of crop species by the transfer of agronomically relevant traits (Glimelius et al. 1991). However, somatic hybrids obtained between phylogenetically remote species are often sterile and morphologically abnormal, and also show uncontrolled instabilities with parts of one or both genomes being lost during the in vitro passage (Negrutiu et al. 1989a). Asymmetric hybrids carrying the complete genome of the recipient and only a few chromosomes, or chromosome fragments, from the donor genome represent more valuable material (Hinnisdaels et al. 1994). Methods have been developed to try to direct and control the process of chromosome elimination in order to obtain asymmetric fertile hybrid plants. One of the most widely used current method to achieve partial genome or cytoplasm transfer has been to irradiate the donor protoplasts with a high dose of X- or gamma-rays (ionizing irradiation) prior to fusion. These so called donor-recipient fusions have been used to produce asymmetric hybrids between many species in both intra- and intergeneric combinations (Hinnisdaels et al. 1994). Although it is well documented that irradiation leads to a preferential loss of donor DNA (Negrutiu et al. 1989b) the genetic make up of asymmetric somatic hybrids is very variable. In a number of fusion combinations large amounts of donor DNA are retained (Imamura et al. 1987; Gleba et al. 1988; Famelaer et al. 1989; Wijbrandi et al. 1990a,b,c; Wolters et al. 1991; McCabe et al. 1993) while in others chromosome elimination has been more thorough leading to highly asymmetric lines (Dudits et al. 1987; Bates et al. 1987; Bates 1990; Hinnisdaels et al. 1991). Since increasing radiation dose causes increasing amounts of chromosomal damage one would expect that the extent of elimination of donor chromosomes would increase with irradiation dose. Such a dose-dependent increase in donor chromosome elimination has indeed been demonstrated (Melzer and O'Connell 1992; Kovtun et al. 1993) but numerous other studies reveal little increase in chromosome elimination beyond that induced by a relatively low threshold radiation dose (Wijbrandi et al. 1990c; McCabe et al. 1993, Trick et al. 1994). Chromosome elimination was far more important in intergeneric (Hinnisdaels et al. 1991; 1994) and in interfamily

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combinations (Somers et al. 1986; Dudits et al. 1987). At present, the exact processes underlying chromosome elimination are not known and very little information is available on the effects that the irradiation doses used in somatic cell hybridization experiments exert on protoplast DNA (Schoenmakers et al. 1994). Due to the absence of a trivial "dose-effect relationship" the irradiation dose seems to be of minor importance whereas the taxonomic distance between the fusion partners may play a more important role in chromosome elimination (Derks et al. 1992; Gillissen et al. 1992a,b). The final outcome of a novel combination remains unpredictable and depends on additional factors such as chromosome number, chromosome morphology, cell-cycle kinetics etc. As an alternative and/or supplement to ionizing radiation, UV radiation has been proposed for asymmetric hybridization experiments (Hall et al. 1992a). While both UV and ionizing radiations induce a broad spectrum of physical and chemical modifications in plant and animal DNA, the physiological consequences of UV treatment are much more immediate than those of gamma irradiation. Moreover, the degree of DNA damage observed following UV irradiation was clearly extensive while gamma irradiation at the same biological doses resulted in considerably less DNA damage (Hall et al. 1992b). The number of double-strand breaks in the DNA was found to be proportional to the UV dose applied. So far only one abstract is available on the production of highly interspecific asymmetric hybrids using UV-irradiated donor protoplasts (Atanassov et al. 1991).

In the present paper we report the recovery of highly asymmetric hybrids following fusion of *Nicotiana plumbaginifolia* leaf protoplasts with UV-irradiated kanamycin-resistant leaf protoplasts of *Lycopersicon esculentum*. Hybrids were recovered by selection for kanamycin resistance and the hybrid regenerants were analysed at molecular, cytological and genetic levels.

Material and methods

Plant material

Wild-type *N. plumbaginifolia* (P_2) (2n=2x=20) was used as the recipient plant material. A transgenic clone of *L. esculentum* (2n=2x=24), derived from the line ATW-291 which carried a single copy of the NPT-II gene under the control of the *nos* promoter (Van den Elzen et al. 1985), was used as a donor for the protoplast-fusion experiments.

Isolation, fusion, and culture of protoplasts

Leaf protoplasts from both species were isolated from 4 to 5 week-old aseptically grown plants in an enzyme solution of 1.2% cellulase R-10 (Onozuka) and 0.7% macerozyme R10 during 15 h at 24°C in the dark, as described by Negrutiu et al. (1986). Before fusion, a monolayer of *L. esculentum* donor protoplasts was irradiated in open Petri-dishes for 80 s by a UV source (germicidal 15 W Sylvania lamp). The dose rate was determined to be 25 erg · cm^{-2} · s^{-1}. To prevent photoreactivation, the next steps of protoplast culture were performed in dark conditions.

The density of the protoplasts was adjusted to 1.5×10^6 per ml and the fusion of recipient and irradiated donor cells, mixed in 1:1.5 ratio, was induced by adding PEG 6000 (20% final concentration) for 20 min according to the protocol II described by Hinnisdaels et al. (1991). As control experiments, parental protoplasts were fused without UV treatment or were simply mixed without fusion treatment.

The protoplasts were initially cultivated in liquid K3 medium and subsequentially diluted in MDn medium in the presence of 25 mg/ml of kanamycin monosulphate (Negrutiu et al. 1986). After 4 weeks, the visible calli were transferred on agar-solidified medium supplemented with 50 mg/l of kanamycin monosulphate to allow the selection of hybrid clones.

Kanamycin-resistant calli were subsequently regenerated according to Installé et al. (1985) in selection conditions (50 mg/l kanamycin monosulphate). The absolute fusion frequency (AFF) was determined as the number of growing colonies in selection medium divided by the total number of parental protoplasts.

Cytology

Metaphase plates were prepared according to the method of Pijnacker and Ferwerda (1984). Briefly, young root tips from in vitro-propagated kanamycin-resistant hybrid plants were pre-treated with alpha-bromonaphthalene (0.2% for 30 min) and subsequently fixed in an ethanol/acetic acid mixture (3:1). After dehydration, the root tips were digested for 9 min in an enzyme solution containing 0.5% Pectolyase and 1% Cellulase and the cells were spread in a drop of 60% acetic acid. After air drying, the slides were hydrolyzed in 5N HCl for 20 min, rinsed under tap water and subsequently stained with 2% Giemsa. Approximately 5–10 metaphase plates were analysed per plant.

Enzymatic assay for neomycin phosphotransferase (NPTII) activity

A dot-blot enzyme assay was carried out on hybrid regenerants according to McDonnell et al. (1987).

PCR amplifications

The following two primers (primer 1: GTTCTTTTTGTCAAGACCGACC and primer 2: CAAGCTCTTCAGCAATATCACG) were used for the amplification and detection of the Tn5 kanamycin gene in the hybrid regenerants. PCR amplifications were performed in a 25-µl reaction volume containing 75 mM Tris-HCl pH 9.0, 20 mM ammoniumsulphate, 1.5 mM $MgCl_2$, 200 µM each of dCTP, dATP, dGTP, dTTP (Pharmacia), 300 ng primer and 0.2 units of Goldstar DNA polymerase (Eurogentec), and 10 ng of total genomic DNA. The reaction mixtures were overlaid with mineral oil and subjected to PCR amplification in a Techne PHC-3 machine programmed for 35 cycles of 60 s 95°C, 60 s 55°C and 60 s 72°C. A 72°C incubation for 10 min was included as a final step. The PCR products were separated on a 1% agarose gel.

Total DNA extraction and Southern blotting

DNA was isolated from young leaves of hybrid plants and both parents according to Dellaporta et al. (1983); 10 µg of DNA was digested with either *Eco*RV, *Hin*dIII or *Eco*RI/*Bam*HI according to the instructions of the manufacturer. Electrophoresis, Southern blotting and probe labelling were performed as described by Maniatis et al. (1982). Filters were hybridized at 65°C in a polyethylene glycol-based hybridization solution for 16 h and subsequently washed in 2×SSC, 0.1% SDS at room temperature for 15 min, followed by two washes at 65°C in 0.5–0.2× SSC, 0.1% SDS for 20 min each, and finally put in Saran wrap and autoradiographed.

Hybridization probes

Three types of probe have been used to identify the hybrid regenerants and their parents. For detecting the kanamycin gene a *Eco*RI/*Sal*I fragment of the plasmid pLGVneo 1103 was used. For detecting ribosomal DNA sequences a maize cDNA clone (670 bp) containing part of the 18*s* rRNA was kindly provided by Dr. N. Barbacar (University of Moldavia, Kishinev). For detecting tomato DNA present in the hybrids a *Hin*dIII fragment of 452 bp (pTHG2) from the genome of *L. esculentum* was kindly provided by Dr. P. Zabel (Agricultural University Wageningen, Netherlands)

Results

Selection and regeneration of asymmetric hybrids

Preliminary studies were performed to determine the dose of UV irradiation to be used during fusion experiments. *L. esculentum* protoplasts were submitted to different dosages – 250–4500 erg cm^{-2} – of UV irradiation (exposure times varying between 10 and 180 s). At a dose of 2000 erg cm^{-2} (80 s of irradiation) the division of tomato protoplasts was completely inhibited. This dose was used in the fusion experiments described below.

Five independent fusion experiments have been performed in which donor protoplasts of *L. esculentum* were UV irradiated at the determined dose and fused with the recipient protoplasts of *N. plumbaginifolia* in a recipient:donor ratio of 1:1.5. Protoplast culture and regeneration were performed in conditions defined as optimal for *N. plumbaginifolia* (Installé et al. 1985; Negrutiu et al. 1986).

Three to four days after protoplasts fusion the first cell divisions could be observed and after 3 weeks in culture the colonies were diluted in liquid medium containing kanamycin monosulphate. After 30–40 days of culture in selective medium, 71 kanamycin-resistant putative hybrid calli were selected. This represents an absolute fusion frequency of 5.4×10^{-4} (Table 1).

From these stable resistant clones, 32.8% showed a regeneration capacity. The morphogenetic calli gave rise to numerous shoots (1–20 per callus) but their further development required a longer period of time than in the case of *N. plumbaginifolia* protoplast-derived calli. Hybrids showed typically poor root growth, 3–4 weeks being necessary to develop a lateral root system. In total, 22 clones of kanamycin-resistant regenerated plants were cultured in vitro. Only those plants with a well developed root system were transferred to the greenhouse. From the control experiment, without UV irradiation, only two growing calli were obtained and only one of them showed a regeneration capacity. Due to slow development of the shoot obtained no analysis was possible on this somatic hybrid. In the control experiment, where parental protoplasts were simply mixed without undergoing fusion treatment, no kanamycin-resistant colonies were recovered.

Morphology of the regenerated plants

Phenotypically all regenerants displayed a recipient partner-like morphology, although their growth was less vigorous than the wild-type, which resulted in a smaller size of the somatic hybrids (Fig. 1a). At the rosette stage, some of the hybrids showed multiple rosette formation (Fig. 1b). Leaf morphology appeared to be different, hybrids having smaller, thick and curled leaves which could easily be detached from the plant (Fig. 1c).

Interestingly, anomalies in flower structure were also observed. The incision of the petals was less deep compared to *N. plumbaginifolia* wild-type flowers (Fig. 2a) and in a few cases only four incisions, instead of five, could be observed. Ovaries of hybrid plants appeared swollen (Fig. 2a). In some hybrids a limited number of flowers showed abnormalties in the structure of the stigma (double stigma) (Fig. 2b). Stigmas extruding out of the petals (Fig. 2c) were also noticed.

Among these regenerated plants, seven were considered for further analysis (Table 1).

Analysis of the regenerated plants

Cytological analysis

The morphology and size of the metaphase chromosomes of both fusion partners are clearly distinguishable. All *N. plumbaginifolia* chromosomes are telocentric except for one pair that is subtelocentric (Mouras et al. 1986), whereas

Table 1 Results of five independent fusion experiments between leaf protoplasts of *N. plumbaginifolia* (N.p.) and UV-irradiated leaf protoplasts of *L. esculentum* (L.e.). The UV dose used on tomato protoplasts was 2000 erg · cm^{-2}

Experiment number	Number of parental protoplasts ($\times10^6$) used for fusion		Number of calli growing on selective medium	AFF (10^{-4})	Number of regenerated plants on selective medium	Number of analysed hybrids (FPE/xx)
	N.p.	L.e.				
I	1.2	1.8	8	6.7	3	01, 02, 03
II	2.2	3.3	2	0.9	1	
III	3.6	5.4	17	4.7	2	05
IV	2.7	4.0	30	11.0	13	07, 18
V	3.6	5.4	14	3.9	3	22
Total	13.3	19.9	71	5.4	22	7

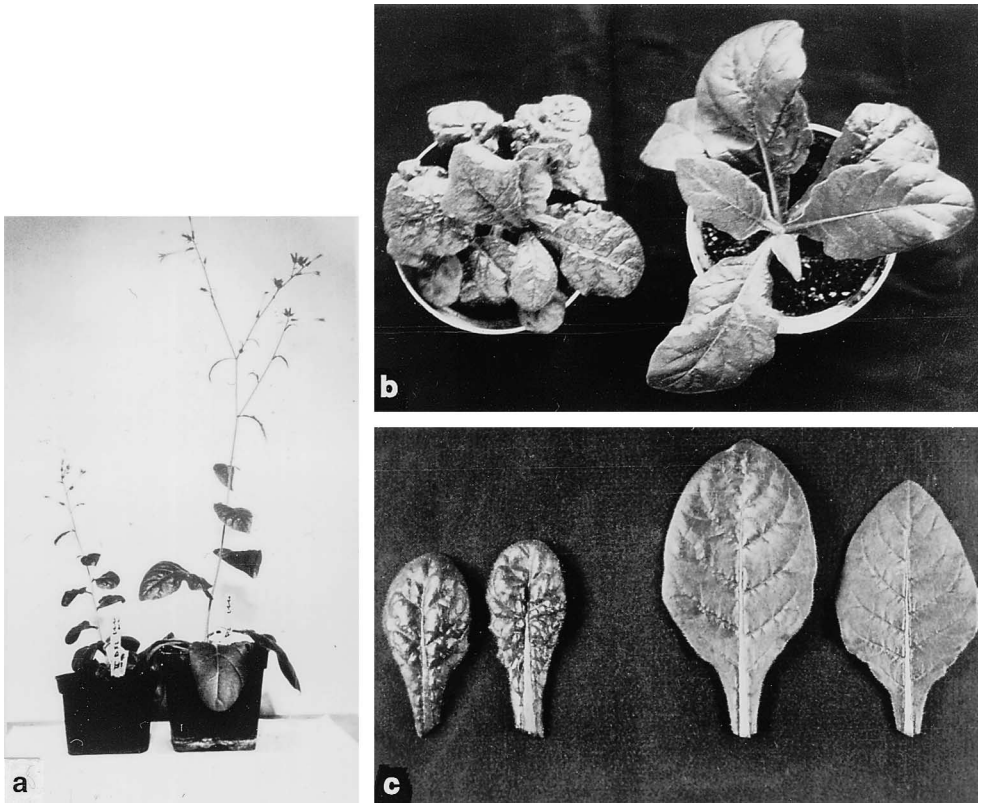

Fig. 1a–c **a** Regenerated plants at flowering stage. **b** Regenerated plants at rosette stage. **c** Comparison of leaf morphology. In each case on the left the asymmetric hybrid FPE/02, on the right the *N. plumbaginifolia* wild-type

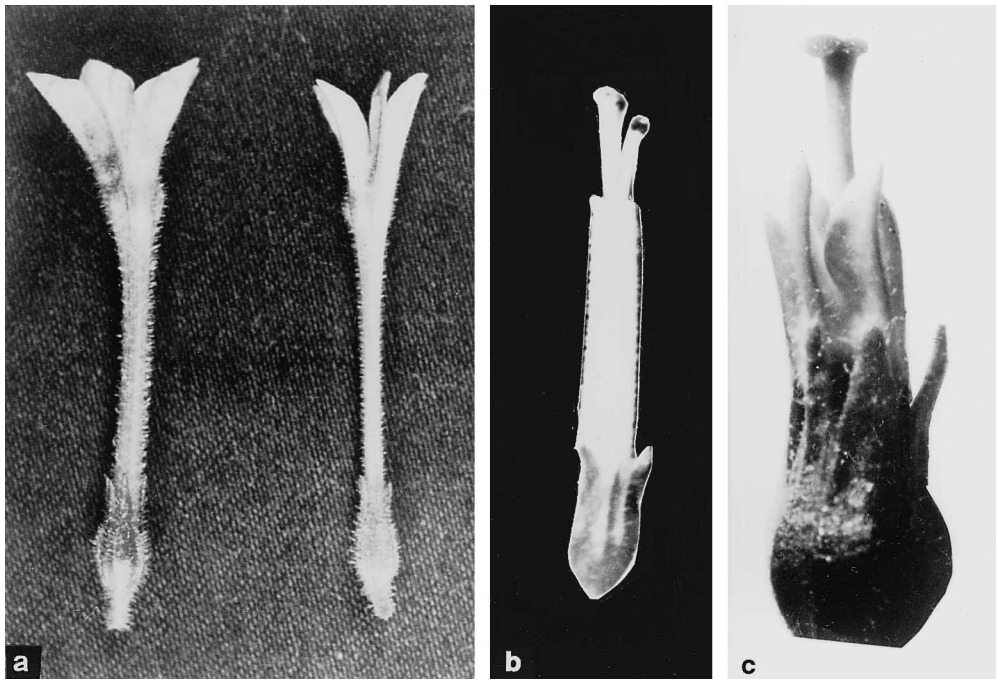

Fig. 2a–c **a** Flower shape of the asymmetric hybrid FPE/01 (left) and *N. plumbaginifolia* (right). **b** Development of an abnormal double stigma in asymmetric hybrid FPE/01. **c** Abnormal stigma extruding out of the petals in asymmetric hybrid FPE/22

tomato chromosomes are submetacentric or metacentric and much smaller (Fig. 3a,b). Data on the karyological analysis of five plants are presented in Table 2. They all possessed *Nicotiana* chromosomes at the polyploid level (mainly hexaploid) as well as 2–4 small tomato chromosome fragments (Fig. 3c,d).

Biochemical and molecular analyses

The *L. esculentum* donor partner carries a functional neomycin phosphotransferase (NPTII) gene, under the control of the Nopaline-synthase promoter, which confers resistance to kanamycin monosulphate (Van den Elzen et al.

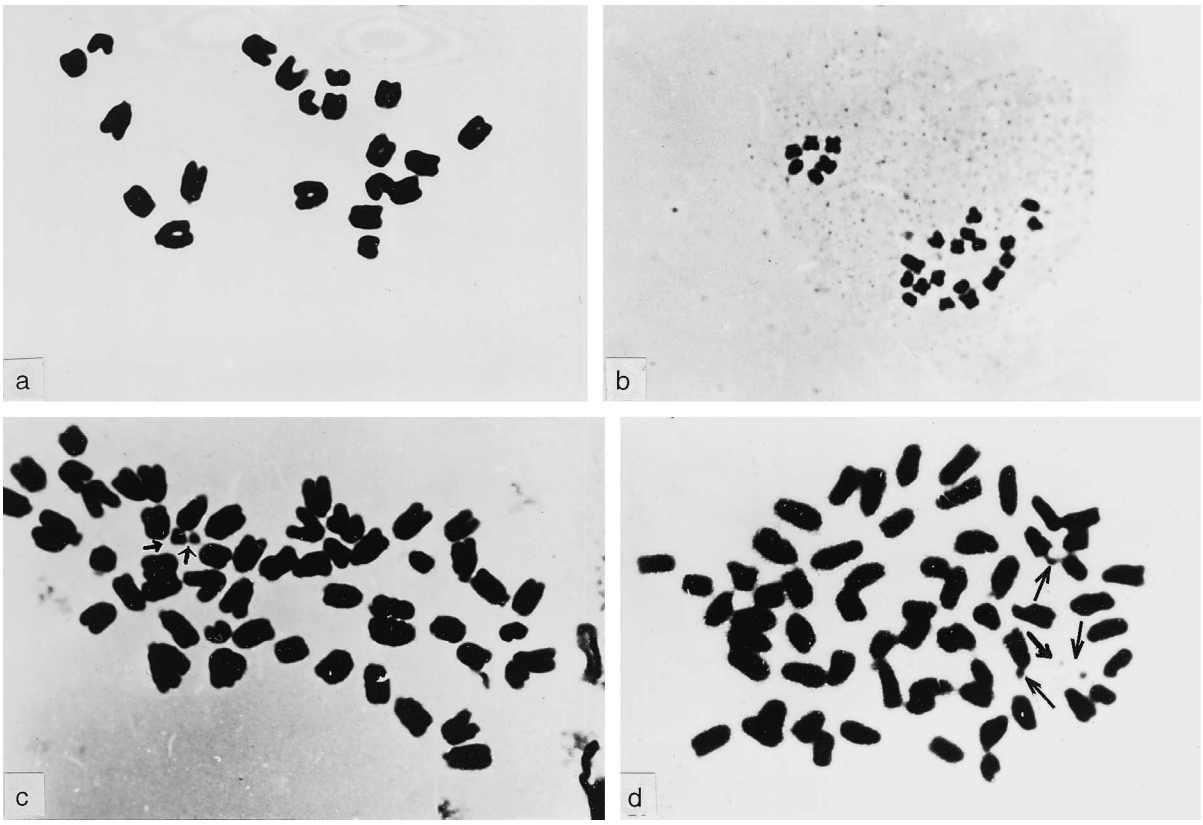

Fig. 3a–d Metaphase plates of *N. plumbaginifolia*, *L. esculentum* and some of their asymmetric hybrids. *L. esculentum* chromosomes or chromosome fragments are indicated by arrows. **a** *N. plumbaginifolia*. **b** *L. esculentum*. **c** Asymmetric hybrid FPE/02. **d** Asymmetric hybrid FPE/03

Table 2 Karyological analysis of regenerants of asymmetric hybrids between *N. plumbaginifolia* and *L. esculentum*

Asymmetric hybrid	Number of *Nicotiana* chromosomes	Number of *Lycopersicon* chromosomes
FPE/02	39–62	3–4
FPE/03	60–72	3–4
FPE/05	40–46	3–4
FPE/07	54–60	2–3
FPE/22	40–60	2–4

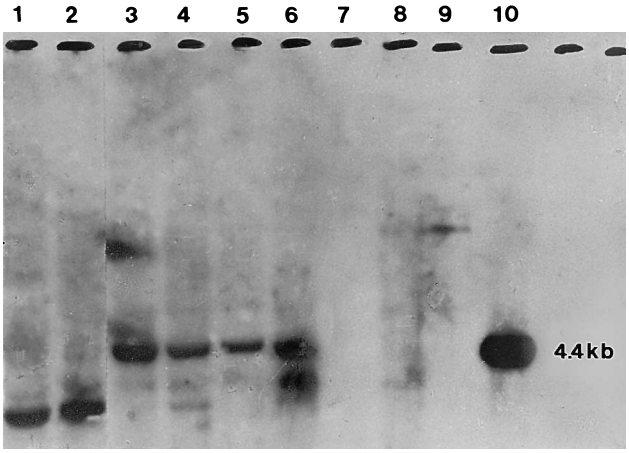

Fig. 4 Southern blot with the kanamycin gene on *Nicotiana*+tomato asymmetric hybrids and their parents. Total genomic DNA was digested with *Eco*RI and *Bam*HI and probed with an *Eco*RI/*Sal*I fragment of plasmid pLGVneo1103, which corresponds to the neomycin phosphotransferase gene. *Lane 1* FPE/01, *2* FPE/02, *3* FPE/05, *4* FPE/07, *5* FPE/18, *6* FPE/22, *8 N. plumbaginifolia*, *10 L. esculentum*

1985). Although all hybrids were permanently grown on basal medium supplemented with kanamycin monosulphate, the presence and functionality of the kanamycin resistance gene was demonstrated by biochemical and molecular analysis. Dot-blot analysis on leaf extracts of the hybrids detected NPTII activity in all hybrids tested (data not shown). Polymerase chain reaction (PCR) amplifications on total genomic DNA isolated from hybrid regenerants using a primer pair specific for the Tn5 kanamycin resistance gene showed the presence of this gene in all the hybrids tested. No amplification product was obtained in the negative control samples (data not shown). Final evidence for the presence of the NPTII (kanamycin resistance) gene in the hybrids was obtained by Southern hybridization. Total genomic DNA from six hybrids was digested with *Eco*RI/*Bam*HI and subsequently hybridized with the *Eco*RI/*Sal*I fragment from the plasmid pLGVneo1103 which contains the Tn5 kanamycin resistance gene. The

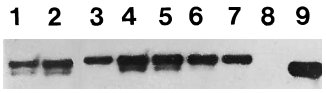

Fig. 5 Southern blot with rRNA sequences on *Nicotiana*+tomato asymmetric hybrids and their parents. Total genomic DNA was digested with *Eco*RV and probed with the maize cDNA clone (670 bp) containing part of the 18*s* rRNA. *Lane 1* FPE/01, *2* FPE/02, *3* FPE/05, *4* FPE/07, *5* FPE/18, *6* FPE/22, *7 N. plumbaginifolia*, *9 L. esculentum*

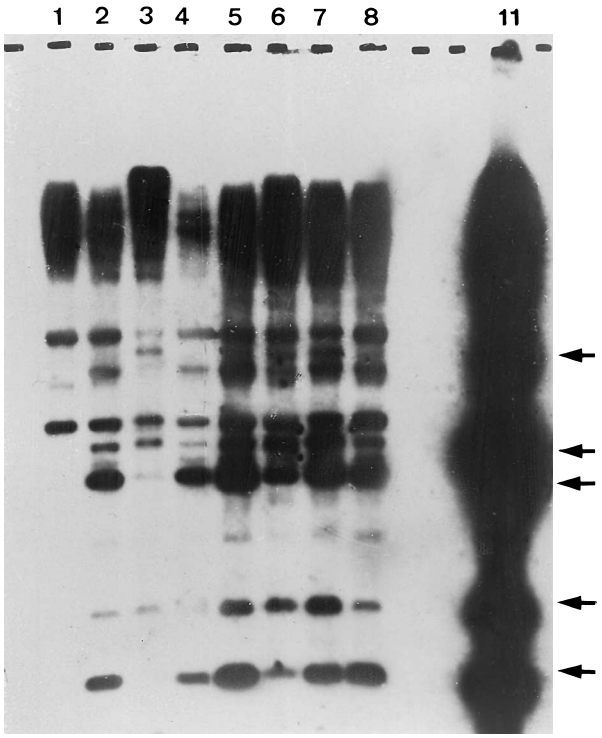

Fig. 6 Southern blot with the tomato repeat pTHG2 on *Nicotiana*+tomato asymmetric hybrids and their parents. Total genomic DNA was digested with *Hin*dIII and probed with a tomato-specific probe: the *Hin*dIII fragment of 452 bp (pTHG2) from the genome of *L. esculentum*. *Lane 1 N. plumbaginifolia*, *2* FPE/01, *3*. FPE/02, *4* FPE/03, *5* FPE/05, *6* FPE/07, *7* FPE/18, *8* FPE/22, *11 L. esculentum*. Specific tomato bands are marked by *arrows*

autoradiogram in Fig. 4 shows that all hybrids tested contain the kanamycin gene. Four of the hybrids (FPE/05, FPE/07, FPE/18, FPE/22) produce a single hybridization band of 4.4 kb which is identical to the fragment obtained in the donor partner *L. esculentum*. Interestingly, two of the hybrids (FPE/01 and FPE/02) produce a hybridization profile that differs from the one observed in the donor partner. The most plausible explanation for this is that intergenomic recombination took place between the two genomes.

Further proof for the hybrid nature of the obtained regenerants came from Southern-hybridization experiments using either rDNA or a tomato-specific repeat as probe.

Total genomic DNA of six hybrids and their parents was digested with *Eco*RV and probed with a partial maize cDNA clone containing part of the 18*s* rRNA gene. The autoradiogram is shown in Fig. 5 and illustrates that four (FPE/01, FPE/02, FPE/07, FPE/18) of the six hybrids tested contain the tomato 18*s* rRNA gene. In hybrid FPE/22 a very faint band, corresponding to the tomato rRNA band, could be observed after prolonged exposure.

The plasmid pTHG2 contains a 452-bp HindIII fragment of *L. esculentum* "moneymaker". This sequence is highly repeated and dispersed in the tomato genome (approximately 7.5×10^4 copies) and provides a powerful tool to detect traces of tomato DNA present in the highly asymmetric hybrids obtained. The results of Southern-hybridization experiments using this tomato repeat as a probe are shown in Fig. 6. Although the probe does cross-hybridize with *Nicotiana* DNA a number of tomato-specific bands are produced on *Hin*dIII-restricted total DNA. All seven hybrids tested contain most of these tomato-specific bands which clearly indicates the hybrid nature of the regenerants. No correlation could be found between the number of tomato chromosomes and the presence and/or absence (intensity) of some of these specific bands.

Progenies of asymmetric hybrids

Hybrid regenerants were grown to maturity in the greenhouse. While most of the plants produced flowers, they were largely male sterile. Seeds from self pollinations were only obtained from two hybrids (FPE/01 and FPE/02). Back crosses with pollen from wild-type *N. plumbaginifolia* produced seeds, but still less when compared with wild-type. Progeny seeds were sown on basal medium Mn (Negrutiu et al. 1983) with or without 50 mg/l of kanamycin monosulphate. Germination occurred at a very low rate (0–3%) even on basal medium and only after treating the seeds by imbibition in gibberelic acid (10 mg/ml during 1 h). For two back-cross progenies (FPE/01×N.p. and FPE/02×N.p.) we recovered a few plantlets (less than ten) after germination. They all showed very slow development on selection medium and most of them persisted as small rosettes in vitro. Two of them which developed further did not survive transfer to the greenhouse.

Discussion

The ultimate objective of asymmetric cell hybridization is the selected transfer of desired traits, chromosomes or chromosome fragments, while minimizing the contribution of the donor genome. The treatment of donor protoplasts with lethal doses of irradiation (X- or gamma-rays) prior to fusion, in combination with strong selection for the gene of interest, has so far been the method of choice for the production of asymmetric nuclear hybrids (Hinnisdaels et al. 1994). Unfortunately, in the majority of cases the hybrids produced contain much more donor DNA than desired, have an aberrant morphology, and are mostly partially sterile (Gleba et al. 1988; Famelaer et al. 1989; Wolters et al. 1991;

Bauer-Weston et al. 1993; Kovtun et al. 1993; McCabe et al. 1993; Trick et al. 1994). Highly asymmetric fertile hybrids have only occasionally been described (Dudits et al. 1987; Bates 1990; Hinnisdaels et al. 1991).

At present the exact mechanisms underlying chromosome elimination are unknown, and due to the absence of a trivial dose-effect relationship the final outcome of a novel combination can not be predicted. Additional factors, such as culture and selection conditions, chromosome number and morphology, cell cycle kinetics and phylogenetic distance, may also play a prominent role in chromosome elimination (Derks et al. 1992). Several alternatives for directed chromosome elimination, such as the use of metatrexate (Dudits et al. 1987) and the use of microprotoplasts (Ramulu et al. 1993), have also been proposed. It is clear that further investigations into the determinants responsible for nuclear elimination in somatic cells will be needed in order to fully exploit asymmetric cell hybridization technology to produce morphologically normal and fertile hybrids on a regular basis. Of interest in this context are experiments that focus on the immediate effects that irradiation, at the doses typically used in asymmetric cell-hybridization experiments, exert on protoplast DNA (Schoenmaekers et al. 1994). As an alternative to ionizing irradiation the effects of UV irradiation has been studied by Hall and coworkers (1992a, b). These authors concluded that the degree of DNA damage observed after UV irradiation was much more pronounced when compared to equivalent biological doses of gamma rays and, therefore, could be used as a valuable alternative or supplement in asymmetric cell hybridization experiments. With the exception of an abstract by Atanassov et al. (1991) no detailed results have been published using UV irradiation in fusion experiments. We have used UV irradiation in order to produce highly asymmetric intergeneric nuclear hybrids between *N. plumbaginifolia* and a kanamycin-resistant *L. esculentum* line. Gamma-fusion experiments with an irradiation dose of 100 krad (Cobalt 60) between these two partners occasionally gave rise to highly asymmetric kanamycin-resistant fertile hybrid plants. With the exception of the kanamycin-resistant gene no traces of tomato DNA could be detected in these regenerants (Hinnisdaels, unpublished). In contrast, by using UV-treated (2000 ergs $\cdot$ cm^{-2}) tomato protoplasts prior to fusion, kanamycin-resistant calli that could be regenerated into hybrid plants were systematically produced, although the frequency of the clones obtained was significantly lower when compared to data from other asymmetric cell-hybridization experiments. The regenerants resembled the recipient partner *Nicotiana* morphologically but their growth was much slower and abberrations in leaf and flower morphology, with multiple rosette formations, were commonly observed. Cytological analyses demonstrated the presence of a few tomato chromosome fragments in a predominantly polyploid *Nicotiana* background. The hybrid nature of the regenerants was further demonstrated by biochemical and molecular analyses. Southern-blotting experiments, using the kanamycin gene as a probe, not only demonstrated the presence of the gene in all hybrids tested but also revealed that in some cases intergenomic recombination had occurred. Final proof for these types of events has, however, to be demonstrated by in situ hybridization studies on metaphase chromosomes (Parokonny et al. 1992).

Species-specific highly repeated sequences have already proven their utility for the identification of somatic hybrids (Schweizer et al. 1988; Pehu et al. 1990; Itoh et al. 1991) as well as for the estimation of the relative contribution of both parental genomes in several somatic hybrids (Imamura et al. 1987; Piastuch and Bates 1990; Wolters et al. 1991; Kovtun et al. 1993). We used a 452-bp specific tomato repeat in order to demonstrate the presence of tomato-specific DNA in the regenerants. Moreover, a ribosomal DNA sequence from both parents was also detected in some of the regenerants.

In conclusion, highly asymmetric partially fertile hybrids were obtained between *N. plumbaginifolia* and *L. esculentum* using UV irradiation of the donor protoplasts prior to fusion. Our results show for the first time that UV irradiation can indeed be used as a substitute, and/or alternative, to ionizing irradiation for the production of asymmetric hybrids, as proposed by Hall and coworkers (1992a,b). Irradiation (either ionizing or UV) directs the process of chromosome elimination but the extent of it and its final outcome clearly depend on additional factors. Therefore, detailed investigations into the determinants responsible for chromosome elimination are highly desirable. Moreover, it is worthwhile stressing the importance of sexual crosses for obtaining further elimination of donor material in order to stabilize the hybrids and to enhance fertility.

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