Theor Appl Genet (1988) 76:760–766

Intergeneric asymmetric hybrids between *Nicotiana plumbaginifolia* and *Atropa belladonna* obtained by "gamma-fusion"

Y.Y. Gleba, S. Hinnisdaels [1], V.A. Sidorov, V.A. Kaleda, A.S. Parokonny, N.V. Boryshuk, N.N. Cherep, I. Negrutiu [1] and M. Jacobs [1]

Institute of Botany of the Ukrainian Academy of Science, Repina 2, SU-25260 Kiev, USSR
[1] Institute of Molecular Biology, B-1640 St.-Génésius-Rode, Belgium

Received February 29, 1988; Accepted May 8, 1988
Communicated by G. Melchers

Summary. Asymmetric nuclear hybrids have been obtained by fusion of cells from a nitrate-reductase deficient mutant of *Nicotiana plumbaginifolia* (cnx20) and gamma irradiated protoplasts of *Atropa belladonna* (irradiation doses tested were 10, 30, 50 and 100 krad). The hybrid formation frequency following selection for genotypic complementation in the NR function was in the range of 0.7%–3.7%. Cytogenetic studies demonstrated that all hybrid plants tested possessed multiple (generally tetra- or hexaploid) sets of *N. plumbaginifolia* ($n=10$) chromosomes along with 6–29 *Atropa* chromosomes ($n=36$), some of which were greatly deleted. Besides the cnxA gene (the selection marker), additional material of the irradiated partner was expressed in some of the lines, as shown by analyses of multiple molecular forms of enzymes. Surprisingly, rDNA genes of both parental species were present and amplified in the majority of the hybrids. Whenever studied, the chloroplast DNA in the hybrids was derived from the *Nicotiana* parent. Regenerants from some lines flowered and were partially fertile. It is concluded that irradiation of cells of the donor parent before fusion can be used to produce highly asymmetric nuclear hybrid plants, although within the dose range tested, the treatment determined the direction of the elimination but not the degree of elimination of the irradiated genome.

Key words: Asymmetric hybrid plants – Irradiation dose – Protoplast fusion – Molybdenum cofactor cnxA gene product

Abbreviations and definitions

Cp	chloroplast
CsCl/EtBr	cesiumchloride/ethidiumbromide
EDTA	ethyldiamine tris acetate
NR	nitrate reductase
SDS	sodiumdodecylsulphate
"Gamma-fusion"	is a conventional abbreviation for fusion experiments in which one fusion partner (the donor) is inactivated by sub- or lethal doses of irradiation before fusion with the other (the recipient) partner.

Introduction

Numerous attempts to use protoplast fusion technology for hybridization of phylogenetically remote species have so far met with limited success. All interfamily hybrids described to date were genetically unstable and/or morphologically abnormal (Wetter and Kao 1980; Chien et al. 1982 and references therein; Niizeki et al. 1985; Sala et al. 1985), while intergeneric and intertribal nuclear hybrids, though genetically stable, resulted in the formation of teratoma's or abnormal and/or sterile plants only (Gleba and Hoffmann 1979; Krumbiegel and Schieder 1979; Dudits et al. 1980; Gleba et al. 1983, 1986; Potrykus et al. 1984 and references therein).

It has been hypothesized that functional "hybrid" plants between remote species would require an extensive asymmetrization of the fusion products, following either a stepwise loss or a rapid undirectional elimination of chromosomes from one fusion partner (Gleba and Hoffmann 1979).

Experiments of several authors indicated that cybrids, as well as asymmetric nuclear hybrids, can be obtained by fusing normal somatic with irradiation inactivated cells (Zelcer et al. 1978; Aviv and Galun 1980; Menczel et al. 1982, 1983; Gupta et al. 1982; Sidorov et al. 1987). However, information concerning the genetic constitution of such hybrids is still poor. The aim of

our present work was to study the genetic constitution of intertribal hybrids produced by fusion of protoplasts derived from a nitrate reductase-deficient mutant of *Nicotiana plumbaginifolia* (recipient) and wild type *Atropa belladonna* (donor), following irradiation with lethal doses of gamma-rays. The experiments allowed us to assess the effect of the irradiation dose, and the cultural and regeneration conditions, on the obtention of highly asymmetric fertile hybrid plants.

Materials and methods

Plant material

Cofactor deficient molybdenum restorable mutant plants from *N. plumbaginifolia* (cnx20) and the universal hybridizer derivative T116/A1 (NR- and kanamycin resistant, unpublished) were aseptically grown on standard basal medium supplemented with 10 m*M* ammoniumsuccinate and 0.22 m*M* Na_2MoO_4 (Negrutiu et al. 1983). The same basal medium was used to aseptically grow wild type *A.belladonna* plants.

Protoplast isolation, fusion and culture

Mesophyll protoplasts from both parents were isolated as described by Negrutiu et al. (1986). Before fusion, and with the exception of control samples, *A.belladonna* protoplasts were irradiated with gamma-rays (10, 30, 50 and 100 krad) from a $Cobalt^{60}$ source. The fusions were carried out as described by Menczel et al. (1982). Protoplasts were cultured for 2 weeks in liquid K_3 medium and subsequently diluted in MDn medium (Negrutiu et al. 1983, 1986). After 1 month, visible calli were transferred to solid selection medium and regenerated as described elsewhere (Installé et al. 1985).

Isoenzyme analysis

The analysis of multiple molecular forms of enzymes was carried out using electrophoresis in polyacrylamide gels (Davies 1967). Gels were stained according to Brewbaker et al. (1986).

Chromosome analysis

For chromosome preparations, root tips were prefixed in colchicine and fixed in ethanol/acetic acid mixture (3:1). They were subsequently squashed and stained in 1% orcein prepared in 45% acetic acid. Alternatively, metaphase plates were prepared by the method according to Pijnacker et al. (1986).

Nuclear DNA extraction

DNA was isolated according to Shure et al. (1983) from 5–10 g of liquid N_2 frozen leaves with two phenol extractions, precipitation with 2.5 volumes of ethanol and purification on two CsCl/EtBr gradients. After extraction of EtBr with isoamylalcohol, the DNA was extensively dialysed against Tris (10 m*M*) EDTA (1 m*M*) buffer. If DNA concentration was too low, the solution was concentrated against Ficoll 400 and redialysed.

Ribosomal DNA analysis

2–10 µg DNA were digested with EcoR1 or EcoRV (endonucleases were provided by A. Janulaitis) and run on a gel containing 0.7% agarose, 40 m*M* Tris-HCl (pH 7.8), 20 m*M* Na-acetate and 2 m*M* EDTA. The denatured DNA was transferred to nitrocellulose filters (S & S BA85) which, following preincubation at 65 °C for 2–6 h in 4X SET (SET is 0.1 *M* NaCl, 0.03 *M* Tris-HCl pH 7.4, 2 m*M* EDTA), 0.2% SDS, 150 microgram/ml heparin, 5 µl/ml saturated sodium pyrophosphate, were probed with 10^6 cpm of P^{32} labeled plasmid pUC222, containing 500 bp 3-fragment of 25S rRNA gene from *Citrus lemon* (the plasmid was kindley provided by I. Fodor and V. Kolosha). The filters were washed in 2 × SET, 0.2% SDS once and twice in 0.5 × SET, 0.2% SDS at 65 °C, and finally dried and autoradiographed.

Cp DNA isolation and analysis

The cp DNA was prepared from purified chloroplast isolated by a two step sucrose gradient centrifugation. Chloroplasts were lysed and cp DNA was then fractionated by CsCl density gradient centrifugation. Restriction endonucleases SalI and HindIII were used for cp DNA analysis; the fragments were separated by electrophoresis on 0.8% agarose slab gels in Tris acetate buffer.

Embryo rescue

Fruits were collected 3–5 days after pollination, sterilized (10 min in 5% calcium hypochlorite) and dissected (i.e. the integuments were carefully removed). The fruit was cut into two halves and placed with the placenta onto the standard basal medium with 20 g/l sucrose).

Results

1 Hybrid isolation

Numeric data on control and gamma fusion combinations are given in Table 1. The recipient partner (cnx20) and its derivation $T116/A_1$ have a recessive mutation identified as cnx A cofactor function in the NR enzyme complex for which no revertants have been obtained thus far. Fusion products were recovered following selection

Table 1. Data on hybridization between *N. plumbaginifolia* cnx20 and irradiated *A. belladonna* somatic cells

Irradiation dose (krad)	No. of parental cells (× 10^6)		No. of surviving colonies (× 10^4)	No. of colonies on selective medium	Complementation frequency[a] (%)
	Nicotiana	*Atropa*			
10	1.4	1.43	7.8	1.915	0.137 (2.45)
30	1.4	1.43	3.9	1.442	0.103 (3.7)
50	1.96	0.46	1.7	340	0.074 (2.00)
100	1.41	0.87	3.7	263	0.030 (0.71)
Control					
0 (PEG)	0.176	0.176	nd	61	0.035
0 (EF)	0.353	0.353	nd	241	0.061

PEG: polyethylene glycol induced fusion
EF: electrofusion AC 1 MHz-8V (80 V/cm), DC 1650 V/cm 2 × 50 µs
nd: not determined
[a] Frequencies were calculated by dividing the number of selected colonies by the initial number of protoplasts or by the number of surviving colonies (in *brackets*)

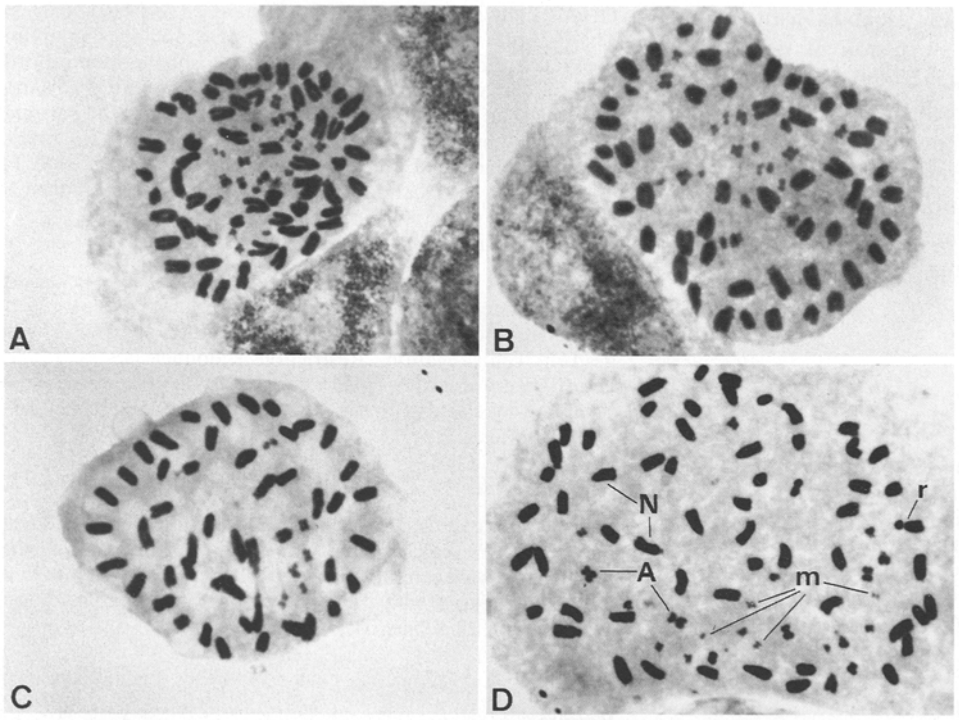

Fig. 1 A–D. Chromosome plates of *Nicotiana plumbaginifolia* + *Atropa* hybrids: **A** clone 1, 10 Krad; **B** clone 12, 30 Krad; **C** clone 16, 50 Krad; **D** clone 13, 50 Krad; typical *Nicotiana* (*N*) and *Atropa* (*A*) chromosomes and reconstructed types (*r*) as well as minichromosomes (*m*) are indicated by *arrows*)

for metabolic complementation in the defective function of the donor partner. Recovery rates for NR^+ colonies varied between 0,7%–3.7%, as calculated from the number of surviving colonies. No clear-cut effect of irradiation dosage on frequency of hybrid formation was registered.

Data in Table 1, obtained after the first selection passage, suggested that the frequencies for metabolic complementation were lower in control non-irradiated samples as compared with the gamma-fusion batches. During subsequent stages of growth, the difference between control and irradiated combinations became more and more obvious. The great majority of the clones produced in control experiments stopped growing and died during the second passage (on solid medium, 10 weeks after fusion). The hybrid nature of few surviving clones was confirmed by chromosome analysis at callus level (data not shown). None of these clones could be regenerated into plants. On the contrary, clones derived from gamma-fusion batches maintained an active growth under selection conditions and regeneration into plant structure was frequently observed (Table 2). Thus, 30%–50% of the stable gamma-fusion products exhibited a regeneration response. Among them, 25%–50% produced relatively normal recipient type regenerants, indicating a rapid, extensive and unidirectional asymmetrization of such fusion products. Only those clones that exhibited regeneration ability were analysed further and are described below.

2 Analysis of primary regenerants

2.1 Karyological analysis. For this study root cells of in vitro grown plants were used. Analysis of chromosome sets was greatly facilitated because metaphase chromosomes of the two parents are clearly distinguishable: *Atropa* chromosomes are at least twice as short and markedly thinner than those of *N. plumbaginifolia*; in addition, all *N. plumbaginifolia* chromosomes are telo- or subtelocentric.

Typical metaphase plates in root tip cells of different hybrids are shown in Fig. 1. The karyological analyses are summarized in Table 3. All *N. plumbaginifolia* + *A. belladonna* hybrids studied possessed, with one exception, polyploid (mostly tetraploid or hexaploid) chromosome sets of *N. plumbaginifolia* ($n=10$), i.e. the recipient species. Hybrid metaphase plates also contained small chromosomes, most of which were morphologically undistinguishable from the *Atropa* ($n=36$) ones. Their number varied from 6–29 in different clones, which theoretically corresponds to 15%–45% of the *Atropa* haploid genome.

Chromosome variability within clones was insignificant and could be due to mistakes of counting (Table 3).

Table 2. Regeneration response in various genomic complementation clones of *N. plumbaginifolia* (recipient) + *A. belladonna* (donor) obtained by gamma-fusion

Irradiation dose (krad)	No. of stable clones[a]	Regeneration induction type			Plant phenotype[b]	
		negative	"terratoma"	positive	aberrant	recipient
10	365	139	104	122	92	30
30	313	143	97	73	50	23
50	80	43	23	14	7	7
100	62	42	8	12	8	4

[a] No. of stable clones: two times confirmed on solid selection medium

[b] Plant phenotype: no. of clones and corresponding phenotypes obtained from the calli giving a positive regeneration induction response

Table 3. Caryological analysis of *N. plumbaginifolia* (*Np*) + *A. belladonna* hybrid (*Ab*) plants: dose effect and clonal variation

Clone no. irradiation dose (krad)	Chromosome no.	No. of *Np* chromosomes	No. of *Ab* and modified chromosomes	No. of plates analysed
Dose effect				
1 /10	74–76	56–58	18–19	9
12 /10	84–85	55–57	28–29	6
39 /10	92–95	74–75	18–20	3
46 /10	88–90	68–69	20–21	6
159 /10	67–72	48–52	19–20	5
251A/10	71–76	55–57	16–19	6
12 /30	73–74	49–51	23–24	5
150A/30	64–75	58–63	8–12	15
156 /30	52–56	41–42	11–14	12
200 /30	38–43	34–36	4–7	11
4 /50	76–77	53–60	16–18	4
5 /50	56–58	38–40	16–18	5
13 /50	81–82	55	24–26	4
16 /50	54–55	48–49	6	7
18B/50	88–91	78–79	10–12	4
63A/50	68–70	55–57	13	4
3/100	60–62	40	20–22	8
Subclonal variation[a]				
200A/30	39–41	34	5–7	3
200E/30	42	36	6	3
200H/30	40–43	34–36	6–7	16
200b1/30	42–44	35–36	7–8	11

[a] All regenerants were obtained from the same original callus

There is no apparent correlation between the level of unidirectional species-specific elimination of the irradiated genome and the irradiation dose. Because of the small size of the *Atropa* chromosomes we were not able to quantitatively analyse chromosomal changes within the donor material. It is clear, however, that some of the small chromosomes, identified *prima facie* as corresponding to the *Atropa* type, are deleted chromosomes. A good example supporting this conclusion is clone 13/50 Krad in metaphase plate of which, besides typical *Atropa* chromosomes, numerous minichromosomes were identified (Fig. 1 d, *arrows*). Therefore, the amount of donor genetic material, calculated only on the basis of the number of small chromosomes per cell, is probably an overestimation.

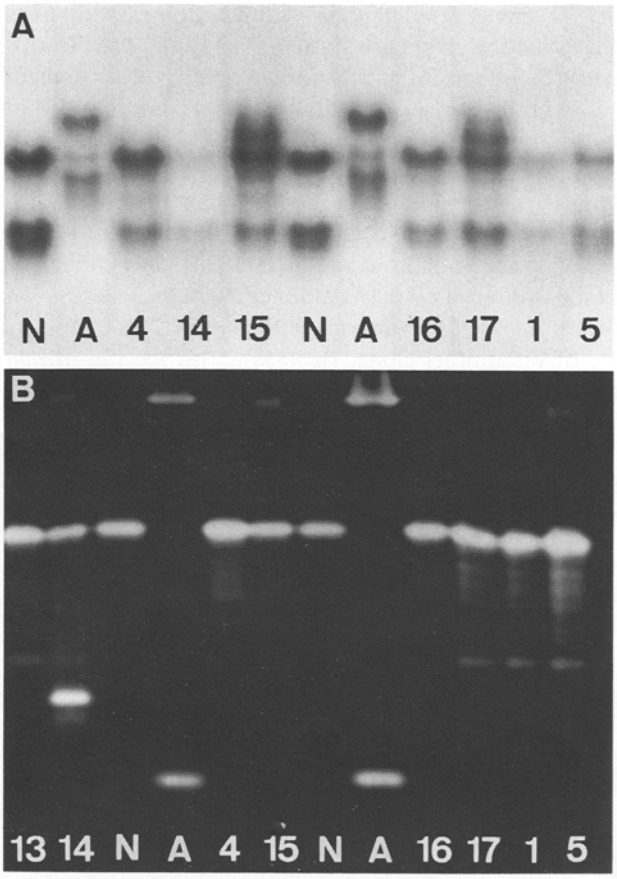

Fig. 2 A und B. Electrophoretic analysis of multiple molecular forms of **A** aspartate aminotransferase and **B** amylase in parental species and *N. plumbaginifolia* + *Atropa* hybrids. The hybrids shown were obtained following irradiation with **A** 50 krad and **B** 10 krad

2.2 Isoenzyme analysis. Whenever tested, the activity of the xanthine dehydrogenase, known to be absent in recipient mutant plants, was restored in fusion products growing under selection conditions (data not shown). Furthermore, 50 hybrid lines were checked for the following isoenzymes: esterase, amylase and aspartate aminotransferase. Only lines 13/30 krad, 15/50 krad and 17/50 krad contained some aspartate aminotransferase molecular forms from the irradiated donor. Typical electrophoretograms are given in Fig. 2.

2.3 Ribosomal DNA analysis. A total of nine hybrid lines were analysed. Data are given in Fig. 3. *Nicotiana* (recipient) rDNA was invariably present in all hybrids studied. In addition, *Atropa* specific fragments were found in 5 hybrid lines (13/10 krad, 12/30 krad, 13/30 krad, 12/50 krad and 3/100 krad): note that the intensity of these bands was in most cases weaker than that of the *N. plumbaginifolia* rDNA fragments. In another 3 lines (11/30 krad, 14/50 krad and 1/100 krad), only either one of the two specific *Atropa* bands could be visualized, even following overexposure of the film.

In yet another clone, 16/50 krad, no specific *Atropa* rDNA repeats could be detected. It should be mentioned that clone 16/50 krad possessed 6 *Atropa*-type chromosomes only, whereas all other clones analysed cytologically (including 12/30 krad and 3/100 krad) had 3 to 5 times more donor chromosomes.

2.4 CpDNA analysis. Restriction endonuclease analysis was performed with 23 hybrid lines. In all cases, the hybrids exhibited the plastid DNA pattern of the recipient species (data not shown).

3 Analysis of progeny plants

The best regenerants identified under in vitro conditions were transferred into the greenhouse. There was some variability in the phenotype of several independently identified asymmetric hybrid products (200/30 krad, 150/30 krad and 18/50 krad): some remained at the vegetative rosette stage (77/10 krad and 251/10 krad), while others produced, at various degrees, abnormal flower structures (150/30 krad). However, all these plants were characterized by a gross *N. plumbaginifolia* phenotype with short or very short habit with extensive lateral branching (bushy-like phenotype).

Clone 200/30 krad (Table 3), which contains 34 *N. plumbaginifolia* + 4–7 *Atropa* chromosomes, was chosen for further testing. Screening for 12 isoenzymes (glutamate dehydrogenase, peroxidase, malate dehydrogenase, 6-phospho gluconatedehydrogenase, glucosephosphate isomerase, shikimate dehydrogenase, malic enzyme, isocitrate dehydrogenase, esterase, hypoxanthine dehydrogenase, glutamate oxaloacetate transaminase and glucose 6 phosphate dehydrogenase) gave no indication of expression of the corresponding *Atropa* genes.

The clone was male sterile, but the backcross to the recipient wild type parent resulted in fruit swelling and abortive seed production, with one exception (progeny 200 DTo × P2) (Table 4).

In embryo rescue experiments following backcross (BC) with diploid and tetraploid *N. plumbaginifolia*, a total of 74 pollinated flowers (54 with the diploid and 20 with the tetraploid male partner) were dissected and cultured and 21 embryo plants were rescued (19 from the diploid and 2 from the tetraploid cross combination). Several of them were grown to maturity: they were male sterile but the phenotype of the BC-1 plants was very similar – although somewhat shorter and more compact – to that of the recipient species *N. plumbaginifolia*.

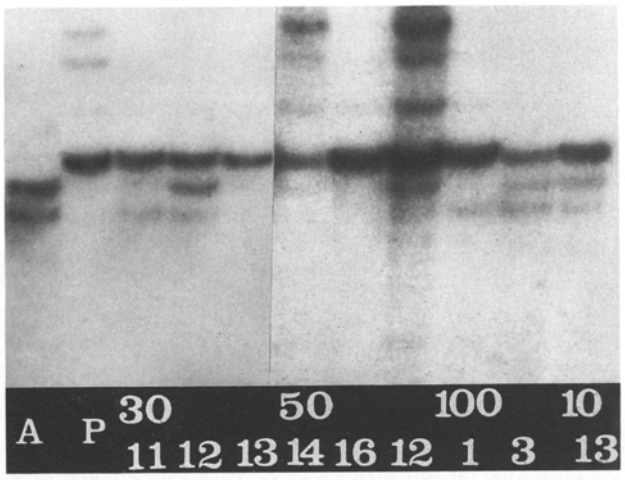

Fig. 3. Autoradiogram of blot-hybridization analysis of ribosomal DNA to digest of DNA from *Atropa* (*A*), *N. plumbaginifolia* (*P*) and their somatic hybrids. *Upper numbers* indicate irradiation doses, *lower numbers* are clone numbers

Table 4. Chromosome number of some F_1 plants obtained by embryo rescue following backcross of asymmetric hybrids to the wild type recipient (for abbreviations see Table 3)

Clone no. irradiation dose (krad)	No. of chromosomes	No. of *Np* chromosomes	No. of *Ab* and modified chromosomes	No. of plates analysed
(200 BT × P_2) T_1B/30	29–31	28–29	1–2	4
(200 BT × P_2) T_1C/30	30–31	28–29	2	2
(200 GT × P_2) T_1 /30	31–32	29	2–3	19
(200 × P_2) T_1B/30	28–29	27	1–2	5
(200 DTo × P_2) /30	31	29	2	6

Flower and leaf morphology were typical and more uniform compared with the Ro-generation. The cytological examination of several progeny plants is detailed in Table 4. It shows that there was a significant reduction of the *Atropa* complement, and that the *N. plumbaginifolia* genome was at (hypo)triploid level (as expected from the hypotetraploid female x diploid male cross combination).

These highly asymmetric triploid hybrid plants were again backcrossed to diploid wild type (BC-2), resulting in improved seed setting and seed germination. This material is under investigation at the present time.

Discussion

Treatment of plant cells with lethal doses of irradiation and subsequent use in fusion experiments was known to create asymmetric nuclear hybrid clones, such as between carrot and parsley (Dudits et al. 1980), *Datura* and *Physalis* (Gupta et al. 1984), *Lycopersicon* species (O'Connell and Manson 1986), several *Solanaceae* (Glimelius et al. 1986; Sidorov et al. 1987), tobacco and barley (Somers et al. 1986), henbane and tobacco (Imamura et al. 1987) and tobacco and carrot (Dudits et al. 1987). Isozymes, species-specific DNA, organelle DNA, immunological assays and chromosomal analysis were employed to measure the level of asymmetry and to show the preferential loss of the irradiated DNA. It results from the cited literature that, at the irradiation doses tested (max 50 krad, usually 10–20 krad), the amounts of nuclear donor DNA retained were relatively large (see Imamura et al. 1987), and only rarely were highly asymmetric clones clearly described (Gupta et al. 1984; Dudits et al. 1980, 1987). Plant structures could be regenerated in many of the described clones, but progeny analysis could be performed only in the carrot and tobacco combination (Dudits et al. 1987). So far, there is no direct proof of gene introgression in plants from protoplast fusion experiments.

We describe here a series of highly asymmetric hybrid plants in an intertribal combination between *N. plumbaginifolia* and *A.belladonna*, obtained after screening large numbers of gamma-fusion products and an extensive caryological investigation of numerous clones, followed by biochemical analysis and genetic assessment of progeny plants. In these experiments, irradiation favoured an unidirectional and relatively rapid (as judged from the ability to regenerate asymmetric plants at the earliest stages of culturing) elimination of most or part of the donor chromosomes. Thus, gamma-fusion appears to be a reliable method for inducing species-specific chromosome elimination from the treated partner in the somatic cell hybrid.

All hybrids obtained in this way were asymmetric and possessed hypohaploid levels of genomic material from the irradiated partner, which corresponded to from 9%–50% of the donor's haploid genome. This conclusion is in rough agreement with the statistical analysis of isozyme markers from the donor partner identified in fusion products: their frequency of transfer varied between 0%–70%. Interesting to note is that most hybrids under investigation possessed multiple copies of rDNAs of both parental species, although the degree of amplification varied greatly among the clones; eventually, the two *Atropa* repeats segregated independently. The frequency of transfer of these genes from an irradiated donor was above 70%. There are several possible explanations of this phenomenon: either there is a strong, selective advantage in the retention of both parental rDNAs (functional implication) or the specific organization of ribosomal genes (specific position on one or several chromosomes, amplification ability, etc.) precludes their inactivation or elimination. This may further imply that the elimination of donor chromosomes is not a fully random process.

One of the most striking findings of our experiments is the fact that the degree of elimination of irradiated donor chromosomes does not depend on the irradiation dose. The hybrids obtained after irradiation with doses as different as 10 and 100 krad all possessed considerable and comparable amounts of donor chromosomal material, whereas the degree of asymmetry in different clones of independent origin, obtained using the same dose, varied more significantly. One can conclude, therefore, that the quantitation of irradiation can not be used to control the process of species-specific chromosome elimination in somatic hybrids. Furthermore, the absence of a trivial "dose-effect" relationship indicated that the elimination process is not due to, or at least not only due to, the mutagenic effect of gamma-rays.

Furthermore, our data show that the donor genome can undergo fragmentation, whose level eventually increases with the dose. However, the number of chromosome breaks per cell is probably relatively low, even at high doses (100 krad). Under the fusion conditions tested, there was a strong selection for centromere within the fusion products, with no evidence so far of alien genome introgression via recombination into the recipient chromosomes. By analysing sufficient independent clones we identified individuals with few donor chromosomes, e.g. highly asymmetric hybrids.

Induction of plant regeneration in such cultures can be considered as a further selection pressure to reveal or accentuate the asymmetry, an observation also made by Sidorov et al. (1987) in *Solanum*. Control experiments with the non-irradiated combination demonstrated the role of the "gamma-asymmetrization" in stabilizing the clones and favouring plant regeneration (also see Gupta et al. 1984).

The regenerated plants exhibited a recipient type phenotype. Among them, fertile individuals (1 out of 64, that is 1.5%, cf. Table 2) were identified among lines with the lowest donor genomic complement and were used to produce progeny plants via backcrossing. This represents a very efficient way to further accelerate and direct the elimination of donor chromosomes, and stabilize the asymmetric hybrid plants while improving fertility.

In conclusion, our results demonstrated that a combination of gamma-fusion, back-crossing and embryo rescue was necessary to by-pass incompatibility barriers in crosses between phylogenetically widely related species to create highly asymmetric hybrid plants and progeny containing a few chromosomes from the donor species.

Acknowledgements. This work was partly supported by grants from IWONL 4972A and 00A 86/91–103. The authors thank Dr. C. Raquin for useful discussions and technical advice on embryo rescue.

References

Aviv D, Galun E (1980) Restoration of fertility in cytoplasmic male sterile *Nicotiana sylvestris* by fusion with X-irradiated *N. tabacum* protoplasts. Theor Appl Genet 58:121–128

Brewbaker JL, Upadkya MD, Makinen Y, MacDonald T (1968) Isoenzymepolymorphism in flowering plants. 3. Gel electrophoretic methods and applications. Physiol Plant 21:930–940

Chien YC, Kao KN, Wetter LR (1982) Chromosome and isozyme studies of *Nicotiana tabacum-Glycine max.* hybrid cell lines. Theor Appl Genet 62:301–304

Davies BY (1967) Disc electrophoresis. 2. Method and application to human serum proteins. Ann NY Acad Sci 121:404–427

Dudits D, Fejer O, Hadlaczky GY, Koncz CS, Lazar G, Horvath G (1980) Intergeneric gene transfer mediated by plant protoplast fusion. Mol Gen Genet 179:283–288

Dudits D, Maroy E, Praznovszky T, Olah Z, Gyorgyey J, Cella R (1987) Transfer of resistance traits from carrot into tobacco by asymmetric somatic hybridization: regeneration of fertile plants. Proc Natl Acad Sci USA 84:8434–8438

Gleba YY, Hoffmann F (1979) *Arabidobrassica:* plant genome engineering by protoplastfusion. Naturwissenschaften 66:547–554

Gleba YY, Momot VP, Okolot AN, Skarzhynskaya MV, Kotov V (1983) Genetic processes in intergeneric hybrids *Atropa* + *Nicotiana.* Theor Appl Genet 65:269–276

Gleba YY, Kanevsky IF, Skarzhynskaya MV, Komarnitsky IK, Cherep NN (1986) Hybrids between tobacco crown gall cells and normal somatic cells of *Atropa belladonna.* Plant Cell Rep 5:394–397

Glimelius K, Fahlesson J, Sjödin K, Sundberg E, Djupsjölacka M, Fellner-Feldegg H, Bonnet HT (1986) Somatic hybridization and cybridization as potential methods for inducing of the gene pools of crops within *Brassicaceae* and *Solanaceae.* In: Horn W, Jensen CI, Odenbach W, Schieder O (eds) Genetic manipulation in plant breeding. Proc Int Symp Eucarpia Berlin. Sept 8-13, 1985. de Gruyter, Berlin New York, pp 663–682

Gupta PP, Gupta M, Schieder O (1982) Correction of nitrate-reductase defect in auxotrophic plants through protoplast mediated intergeneric gene transfer. Mol Gen Genet 188:378–383

Gupta PP, Schieder O, Gupta M (1984) Intergeneric nuclear gene transfer between somatically and sexually incompatible plants through asymmetric protoplast fusion. Mol Gen Genet 197:30–35

Imamura J, Saul MW, Potrykus J (1987) X-ray irradiation promoted asymmetric somatic hybridization and molecular analysis of products. Theor Appl Genet 74:445–450

Installe P, Negrutiu I, Jacobs M (1985) Protoplast derived plants in *N. plumbaginifolia*: improving the regeneration responses of wild type and mutant cultures. J Plant Physiol 119:443–454

Krumbiegel G, Schieder O (1979) Selection of somatic hybrids after fusion of protoplasts from *Datura innoxia* Mill and *Atropa belladonna* L. Planta 145:371–376

Menczel L, Galiba G, Nagy F, Maliga P (1982) Effect of radiation dosage on efficiency of chloroplast transfer by protoplast fusion in *Nicotiana.* Genetics 100:487–495

Menczel L, Nagy F, Lazar G, Maliga P (1983) Transfer of cytoplasmic male sterility by selection for streptomycin resistance after protoplast fusion in *Nicotiana.* Mol Gen Genet 189:365–369

Negrutiu I, Dirks R, Jacobs M (1983) Regeneration of fully nitrate reductase deficient mutants from protoplast cultures of haploid *N. plumbaginifolia.* Theor Appl Genet 66:341–347

Negrutiu I, De Brouwer D, Watts JW, Sidorov VA, Dirks R, Jacobs (1986) Fusion of plant protoplasts: a study using auxotrophic mutants of Nicotiana plumbaginifolia, viviani. Theor Appl Genet 72:279–286

Niizeki M, Tanaka M, Akada S, Hirai A, Saito K (1985) Callus formation of somatic hybrids of rice and soybean and characteristics of the hybrid callus. Jpn J Genet 60:81–92

O'Connell MA, Manson MR (1986) Regeneration of somatic hybrid plants formed between *Lycopersicon esculentum* and *Solanum richii.* Theor Appl Genet 72:59–65

Potrykus I, Jia J, Lazar GB, Saul M (1984) *Hyoscyamus muticus* + *Nicotiana tabacum* fusion hybrids selected via auxotroph complementation. Plant Cell Rep 3:68–71

Pijnacker LP, Walck K, Ferwerda MA (1986) Behaviour of chromosomes in potato leaf tissue cultured in vitro as studied by Bidc-Giemsa labeling. Theor Appl Genet 72:833–839

Sala C, Biasini MG, Morandi C, Nielsen E, Parisi B, Sala F (1985) Selection and nuclear DNA analysis of cell hybrids between *Daucus carota* and *Oryza sativa.* J Plant Physiol 118:409–419

Shure M, Wessler S, Fedoroff N (1983) Molecular identification and isolation of the Waxy locus in maize. Cell 35:225–233

Sidorov VA, Zubko MK, Kuchko AA, Komanitsky IK, Gleba YY (1987) Somatic hybridization in potato: use of gamma-irradiation protoplasts of *Solanum pinnatisectum* in genetic reconstruction. Theor Appl Genet 74:364–368

Somers DA, Narayanan KR, Kleinhofs A, Cooper-Blauds S, Cocking EC (1986) Immunological evidence for transfer of the barley nitrate reductase structural gene to *N. tobacum* by protoplast fusion. Mol Gen Genet 204:296–301

Wetter LR, Kao KN (1980) Chromosome and isoenzyme studies on cells derived from protoplast fusion of *Nicotiana glauca* with *Glycine max-Nicotiana glauca* cell hybrids. Theor Appl Genet 57:273–276

Zelcer A, Aviv D, Galun E (1978) Interspecific transfer of cytoplasmic male sterility by fusion between protoplasts of normal *Nicotiana sylvestris* and x-ray irradiated protoplasts of male sterile *N. tabacum.* Z Pflanzenphysiol 90:397–408