Theor Appl Genet (1991) 82:609–614

Highly asymmetric intergeneric nuclear hybrids between *Nicotiana* and *Petunia*: evidence for recombinogenic and translocation events in somatic hybrid plants after "gamma"-fusion

S. Hinnisdaels[1,*], L. Bariller[2], A. Mouras[2], V. Sidorov[3], J. Del-Favero[1], J. Veuskens[1], I. Negrutiu[1], and M. Jacobs[1]

[1] Free University of Brussels, Institute for Molecular Biology, Paardenstraat 65, B-1640 St-Genesius-Rode Belgium
[2] Université de Bordeaux II, Laboratoire de Biologie Cellulaire, Av. des Facultés, F-33405 Talence-Cedex, France
[3] Institute of Botany, Academy of Science of Ukr.SRR, Division of Cell Biology and Engineering, Lebedeoastr. 1, Kiev GSP22, 252650 USSR

Received October 20, 1990; Accepted February 20, 1991
Communicated by Yu Gleba

Summary. Extremely asymmetric nuclear hybrids have been obtained via protoplast fusion in an intergeneric combination. Irradiated (cobalt^{60}, 100 krad) kanamycin-resistant *Petunia hybrida* mesophyll protoplasts were chemically fused with wild-type mesophyll protoplasts of *Nicotiana plumbaginifolia*. Eighty-six hybrid colonies were selected on kanamycin-containing medium, and twenty-four of these could be induced to regenerate numerous shoots. Cytological analysis of the regenerants showed the presence of a few chromosome fragments in some lines, and even a metacentric chromosome in yet another line. Besides additional chromosome fragments some lines only possessed typical *Nicotiana* chromosomes, and this at the diploid (2n = 2X = 20) as well as the tetraploid (2n = 2X = 40) level. Biochemical analysis showed that all regenerants had neomycin phosphotransferase activity (NPTII), which suggests that intergenomic recombination and or translocation events took place at least in those lines where no additional chromosome fragments could be detected. The presence of the NPTII gene was shown by Southern hybridization. All regenerants tested were fertile, and the segregation ratios for the kanamycin gene (for self and backcross pollinations to the recipient partner) for some of the regenerants correspond with Mendelian rules for a monogenic dominant marker. Most of the regenerants showed abnormal segregation ratios; in this case, no correlation could be made between segregation ratio and chromosome composition.

Our results demonstrate the existence of intergenomic recombination and translocations evens in nuclear somatic hybrid plants obtained via "gamma"-fusion.

Key words: "Gamma"-fusion – Highly asymmetric nuclear hybrids – Intergenomic recombination – Translocation

Introduction

Sexual incompatibility barriers strongly restrict gene exchange between different species. The use of somatic cells as targets in somatic hybridization experiments has opened new possibilities for combining different plant genomes. Moreover, recent advances in plant tissue culture techniques, especially the development of protoplast-regenerating systems in some major crops, has enabled researchers to extend genetic variability by using protoplast fusion technology. As a result, a wide range of hybrid types, with respect to their nuclear and cytoplasmic constitution, has been produced by protoplast fusion (Yang et al. 1988; Tanno-Suenaga et al. 1988; Smith et al. 1989; Sjödin and Glimelius 1989a; Pehu et al. 1989; Han San et al. 1990). In somatic hybrids of remote species, however, various levels of somatic incompatibility reactions become evident (Harms 1983). Fusion products between remote species generally eliminate spontaneously one of the parental genomes thereby creating asymmetric hybrids that contain in addition to a complete recipient genome a few chromosomes derived from the donor. Since one often wants to introduce only a small number of traits from the donor into the recipient, methods for transferring part of the plant genome have been developed. At present, the most frequently used technique for creating asymmetric somatic hybrids is the donor-recipient method – also called "gamma"-fusion. A number of interesting nuclear asymmetric hybrids have been created in this way (Dudits et al. 1980; Gupta et al.

* To whom correspondence should be addressed

1982, 1984; Somers et al. 1986; Gleba et al. 1988; Sjödin et al. 1989 b; Yamashita et al. 1989). From these results it has become clear that while irradiation directs the process of chromosome elimination, it does not control the process single handedly. Indeed, highly asymmetric hybrids containing only one or a few donor chromosomes have only rarely been described, irrespective of the irradiation dose used (Dudits et al. 1980; Bates et al. 1987; Agoudgil et al. 1990).

In the present article we report on the isolation of extremely asymmetric nuclear hybrids after donor-recipient fusions between irradiated kanamycin-resistant *Petunia hybrida* and wild-type *Nicotiana plumbaginifolia* mesophyll protoplasts. Hybrid colonies were easily selected from non-fused cells on culture media supplemented with kanamycin. Hybrid regenerants were analysed at biochemical, molecular, cytological and genetic levels.

Material and methods

Plant material

Wild-type *Nicotiana plumbaginifolia* viviani plants (P_2, $2n=2X=20$) were aseptically grown on basal Murashige and Skoog medium (1962).

Transgenic *Petunia* plants were obtained by leaf disc transformation of a F_1 hybrid of *Petunia hybrida* (Violet 23 × Red 51) using a disarmed *A. tumefaciens* strains harbouring the pTiB6S3::pMON200 cointegrate plasmid as described in Wallroth et al. (1986). Seeds from the backcross of transformant 2828 to the parental line V23 (kindly provided by A. G. M. Gerats, Department of Genetics University of Amsterdam) were sown on basal medium supplemented with 100 mg/l kanamycin monosulphate. Resistant plants were aseptically grown on basal medium supplemented with 75 mg/l kanamycin monosulphate.

Isolation, fusion and culture of protoplasts

Mesophyll protoplasts were isolated from 4- to 6-week-old plants of *N. plumbaginifolia* (P_2) and kanamycin-resistant *Petunia hybrida* (transformant VR2828 × V23) as described by Negrutiu et al. (1986). Before fusion kanamycin-resistant *Petunia* protoplasts were irradiated with gamma-rays (100 krad) from a cobalt^{60} source. Fusions were carried out as described by Menczel et al. (1982). The protoplasts were further cultured in K_3 medium and subsequently diluted in selection medium (MDn) supplemented with 25 mg/l kanamycin monosulphate. After 1–2 months visible calli were transferred to solid selection medium and subsequently regenerated as described by Installé et al. (1985). Control experiments were carried out under the same conditions.

Cytological analysis

For chromosome analysis metaphase plates were prepared using the protoplast method as described by Mouras et al. (1978). Alternatively, metaphase spreads were also obtained from the root tips of regenerated plants as described by Pijnacker and Ferwerda (1984).

DNA analysis

Total genomic DNA from leaf tissue was prepared as described by Shure et al. (1983). The DNA (2–10 µg) was restricted with endonucleases according to the suppliers instructions. Electrophoresis, Southern blotting, Nick translation, hybridization and autoradiography were performed as described by Maniatis et al. (1982). As a probe we used the EcoRI/SalI fragment (representing a complete kanamycin gene) from plasmid pLGVneo1103.

Enzymatic assay for neomycin phosphotransferase activity (NPTII)

The NPTII activity in hybrid regenerants was detected using the in situ gel assay developed by Reiss et al. (1984) adapted for plant tissue as described by Paszkowski et al. (1984).

Genetic analysis

Hybrid regenerants with a well-developed rooting system were transferred into the greenhouse. Self pollinations and backcrosses to the recipient partner *N. plumbaginifolia* were made. Seeds were collected and sown on basal medium supplemented with 100 mg/l kanamycin monosulphate. Segregation ratios for the kanamycin gene were then determined.

Results

Hybrid isolation

One to two weeks after fusion of wild-type *N. plumbaginifolia* (P_2) protoplasts with irradiated (100 krad) kanamycin-resistant *Petunia hybrida* (transformant VR2828 × V23) protoplasts kanamycin monosulphate at a concentration of 25 mg/l was added to the culture medium. After a further culture of 1 to 2 months, green resistant calli could be obtained at a frequency of about 10^{-4}. This transformation frequency is in the range of "gamma"-fusion experiments. In total 86 stable kanamycin-resistant calli were recovered and 24 (28%) could easily be regenerated into plants that resembled the recipient partner *N. plumbaginifolia*. Moreover, organogenic hybrid calli regenerated numerous shoots that were analysed at the cytological, molecular and genetic levels. As expected, control experiments did not result at all in the production of resistant colonies on the selection medium.

Cytological analysis

The analysis of the chromosome composition of the hybrids was greatly facilitated by the fact that metaphase chromosomes of both parents are clearly distinguishable (Fig. 1 A, B). All *N. plumbaginifolia* chromosomes are telocentric except for one pair that is subtelocentric (Mouras et al. 1986), while *Petunia* chromosomes are meta- and submetacentric. Data on the karyological analysis are summarized in Table 1. In total 14 lines have been analysed. Most of them possessed only *Nicotiana* chromosomes at the diploid ($2n=2x=20$) or tetraploid ($2n=4x=40$) level. However, in 5 lines [1 at the diploid (VN12) and 4 nearly at the tetraploid (P_6, VN_6, N_{12}, N_{12}, N_{14}) level] a few (2–3) chromosome fragments

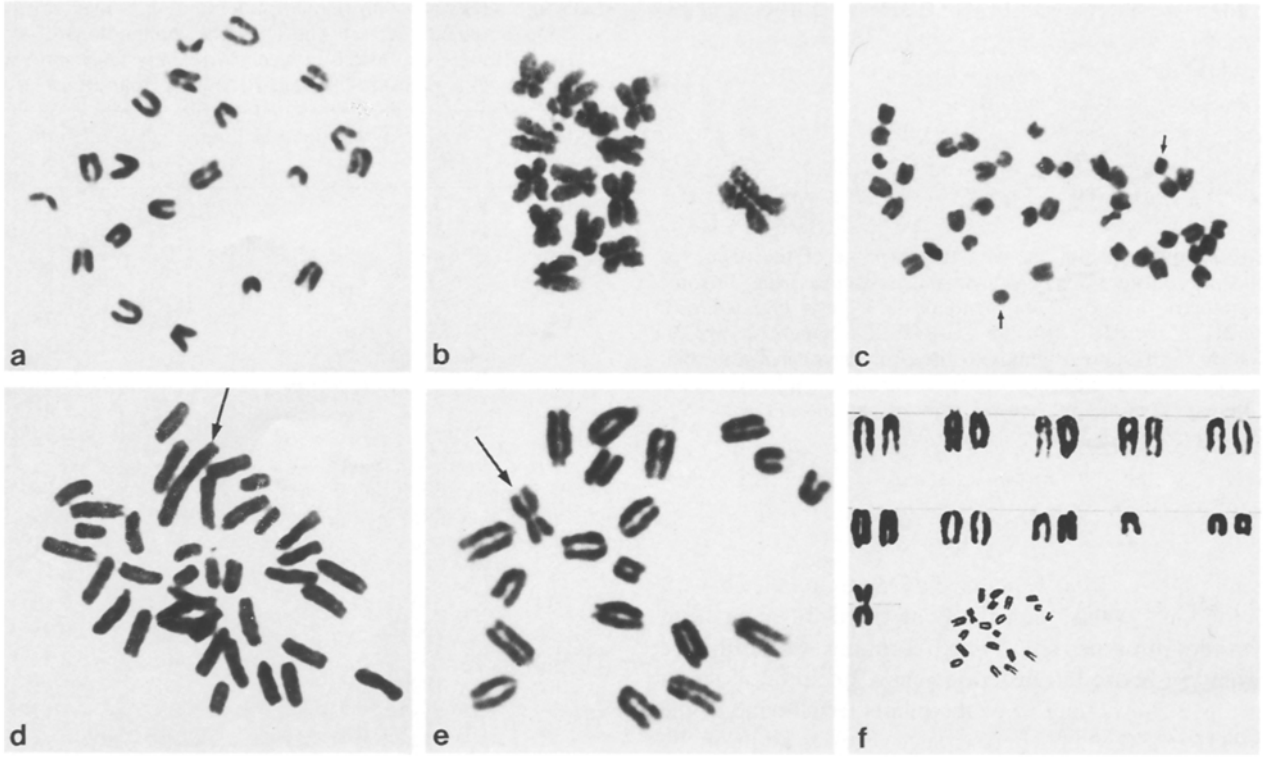

Fig. 1 a–f. Metaphase plates of several *Nicotiana* + *Petunia* hybrid regenerants and their parents. *Petunia* chromosomes or chromosome fragments are indicated by *arrows*. **a** Diploid karyotype of *Nicotiana plumbaginifolia* (P_2); **b** diploid karyotpye of *Petunia hybrida* (kanamycin resistant); **c** line P6; 2-3 chromosome fragments in a nearly tetraploid *Nicotiana* background; **d** line N14; 1 megachromosome in a nearly tetraploid *Nicotiana* background; **e** line P1; 19 *Nicotiana* chromosomes +1 metacentric chromosome; **f** line P1; karyotype according to chromosome length

Table 1. Chromosome composition of hybrid regenerants obtained after "gamma"-fusion between *Nicotiana plumbaginifolia* and kanamycin-resistant *Petunia hybrida* protoplasts

Code	Chromosome number	
$P_2 + V_{23}$	*Nicotiana*	*Petunia* + mini-chromosomes
P_2	20	–
V_{23}	–	14
8, N13	40	–
28, N12	38	1
46, P7	20	–
44, P8	20	–
44, N11	20	–
23, N15	20	–
57, P1	19	1
42, P9	40	–
14, P10	40	–
28, P5a	≈40	–
5, P6	36	1-2
VN12	20	2
VN6	≈40	3-4
14, N14	38	1

–, Not detectable; P_2 *Nicotiana plumbaginifolia* (wild type); V_{23} *Petunia hybrida* (kanamycin resistant)

could be identified (Fig. 1 C, D). In yet another line (P_1) we identified a metacentric chromosome in addition to nearly a complete *Nicotiana* complement (Fig. 1E). Due to the availability of detailed knowledge of the *Nicotiana plumbaginifolia* karyotype (Mouras et al. 1986), we were able to figure out that it is probably 1 chromosome of pair 9 from *Nicotiana* that is missing. However, by observing Fig. 1 F, which shows the karyotype of this P_1 line, one could also speculate that the metacentric chromosome is not a complete *Petunia* chromosome but a translocation of a *Petunia* chromosome piece onto chromosome 9 of *Nicotiana plumbaginifolia*. In order to sort out these two possibilities we are currently analysing this particular line for chromosome pairing during meiosis and also by in situ hybridization.

Molecular analysis

Although all hybrids could be grown and rooted on basal medium supplemented with 50 mg/l kanamycin monosulphate, proof for the presence of the neomycin phosphotransferase gene (kanamycin resistance gene) in these hybrids was obtained by Southern hybridization of ge-

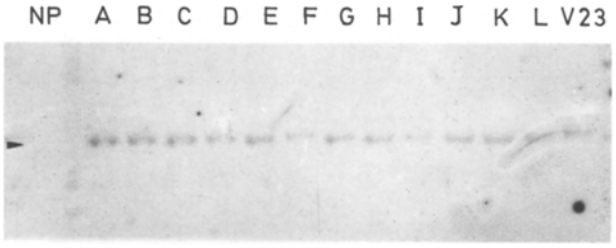

Fig. 2. Southern blot showing the presence of the neomycin phosphotransferase gene (kanamycin resistance gene) in some asymmetric hybrids. Total genomic DNA was digested with EcoRI and probed with the EcoRI/SalI fragment of pLGV-neo1103, which corresponds to the entire neomycin phosphotransferase gene. N_p *Nicotiana plumbaginifolia* wild-type, A-L different asymmetric between *Nicotiana* and *Petunia*, V_{23} *Petunia hybrida* kanamycin resistant

nomic DNA. Total genomic DNA was restricted with EcoRI and probed with the EcoRI/SalI fragment from plasmid pLGVneo1103, which contains the authentic neomycin phosphotransferase gene. The autoradiogram in Fig. 2 shows that all of the plants tested contain the NPTII gene (kanamycin resistance gene). In addition, by using the in situ gel assay on leaf extracts, we showed that these plants contained neomycin phosphotransferase activity (NPTII) (data not shown).

Genetic analysis

All hybrid regenerants with a well-developed rooting system were transferred into the greenhouse and subsequently, self pollinated and backcrossed to wild-type *N. plumbaginifolia*. Seed set for most of the hybrids was good, although somewhat lower than in wild-type *N. plumbaginifolia*. Typical data for segregation of the kanamycin gene in these fusion hybrids are given in Table 2. The gene for kanamycin resistance is inherited as a dominant Mendelian trait. Since the donor partner *Petunia hybrida* is known to contain only one copy of the kanamycin gene (Wallroth et al. 1986), it was expected that self pollination of the hybrids would result in a 3:1 ratio of resistant (R) and sensitive (S) progeny, while a 1R:1S segregation would be expected after backcrossing to the recipient partner *N. plumbaginifolia*. From the data in Table 2 it is clear that while the segregation ratios of some of the hybrids are consistent with the expected ratios most of the hybrids show deviating segregation patterns. No correlation could be found between the segregation pattern and the chromosome composition of the hybrids. The high fertility (self and backcross) of the obtained hybrid regenerants is unique for asymmetric nuclear hybrids and could be explained by the high level of asymmetry of the regenerants in their chromosome composition.

Table 2. Segregations on kanamycin-containing medium of seedlings obtained after self and backcross pollinations of hybrid regenerants from fusions between *Nicotiana plumbaginifolia* and irradiated kanamycin-resistant *Petunia hybrida* protoplasts

Code P_2+V_{23}	Segregation seedlings R		S	Ratio R:S
57, P1	R_1 457		142	3.22:1
	F_1 174		146	1.19:1
48, P2	R_1 1219		34	35.85:1
	F_1	nd		nd
8, P4	R_1 501		23	21.78:1
	F_1	nd		nd
28, P5	R_1 734		21	34.95:1
	F_1 232		45	5.15:1
5, P6	R_1 988		34	29.05:1
	F_1 233		62	3.76:1
46, P7	R_1 397		140	2.84:1
	F_1 203		206	0.99:1
42, P9	R_1 929		18	51.61:1
	F_1	nd		nd
44, N11	R_1 1534		572	2.68:1
	F_1 130		133	0.98:1
23, N15	R_1 1335		401	3.33:1
	F_1 138		117	1.18:1
VN9	R_1 211		8	26.38:1
	F_1 54		7	7.7 :1
VN12	R_1 392		133	2.9 :1
	F_1 246		240	1.02:1
3, P3	R_1 170		6	28.33:1
	F_1 171		28	6.11:1
65A	R_1 28		6	4.67:1
	F_1 33		27	1.22:1
65B	R_1 120		2	60 :1
	F_1 134		30	4.47:1
VN3	R_1 257		11	23.36:1
	F_1 100		28	3.57:1
14, N14	R_1 99		23	4.30:1
	F_1 13		20	0.65:1
14, P10	R_1 274		15	18.17:1
	F_1 70		64	1.09:1
8, N13	R_1 276		7	39.42:1
	F_1 41		12	3.42:1
VN16	R_1 539		20	26.95:1
	F_1 267		56	4.77:1
VN11	R_1 417		20	20.85:1
	F_1	nd		nd
23	R_1 339		88	3.85:1
	R_1 145		132	1.10:1
VN10	R_1 382		16	23.88:1
	F_1 177		35	5.06:1

P_2, *N. Plumbaginifolia;* V_{23}, *Petunia hybrida;* R, resistant; S, sensitive; R_1, self pollination; F_1, backcross pollination to wild-type *N. Plumbaginifolia;* nd, not determined

Discussion

The treatment of plant cells with lethal doses of irradiation and subsequent use in fusion experiments has resulted in the production of a number of asymmetric nuclear hybrid clones (Hinnisdaels et al. 1988). From the results obtained so far one can conclude that irradiation can be used to direct the process of chromosome elimination, but the absence of a trivial dose effect (Gleba et al. 1988) suggests that the elimination process is not only due to the mutagenic effect of the irradiation rays. As a matter of fact, highly asymmetric clones containing only 1–2 donor chromosomes have only rarely been obtained, and this usually after the screening of large numbers of fusion products (Dudits et al. 1980; Gupta et al. 1984; Bates et al. 1987; Agoudgil et al. 1990). Biochemical (isozymes), molecular (species-specific probes, organelle DNA,. . .) and cytological analysis have been the usual means employed to prove hybridity and to show the preferential loss of irradiated donor DNA (Imamura et al. 1987). In many cases plant structures could be regenerated, but progeny analysis has only been reported by Dudits et al. 1987; Bates et al. 1987; Gleba et al. 1988 and Agoudgil et al. 1990. So far little evidence exists for gene introgression or translocation events in plants from protoplast fusion experiments (de Vries et al. 1987; Wijbrandi et al. 1990); Piastuch and Bates 1990).

In the present article we report on the production of completely fertile highly asymmetric nuclear hybrids between *Nicotiana plumbaginifolia* and *Petunia hybrida*. Hybrid selection relied on the presence of kanamycin resistance in the irradiated donor species (*Petunia*). All of the regenerated shoots resembled the recipient partner *N. plumbaginifolia*. The presence of the kanamycin gene in hybrid regenerants has been shown at the biochemical level (NPTII assay) and also by Southern hybridization on genomic DNA. The high fertility (self as well as backcross pollinations to the recipient partner) is unique for "gamma"-fusion hybrids. In a number of the regenerants the kanamycin gene segregated in a Medelian fashion, or with some deviations. Most interesting results were obtianed from cytological analysis. Our detailed knowledge of the karyotype of both fusion partners enabled us to characterize the hybrids precisely. Most of the lines analysed contained only *Nicotiana* chromosomes, which probably reflects their high fertility. In some lines we were able to demonstrate the presence of a few chromosome fragments that were probably created by the irradiation of the *Petunia* protoplasts. In yet another line one clearly metacentric chromosome could be identified in addition to a nearly complete (at least in morphology) *Nicotiana* complement. Indeed, besides the metacentric chromosome only 19 *Nicotiana* chromosomes could be identified. Two possible explanations can be envisaged for this chromosome composition: firstly, the metacentric chromosome is a complete *Petunia* chromosome and secondly, the metacentric chromosome represents a translocation of a chromosome fragment from *Petunia* onto a *Nicotiana* chromosome. To discover what the answer is we are currently analysing this very interesting line for chromosome pairing at meiosis and by *in situ* hybridization. These results might help us to demonstrate the existence of recombinogenic or translocation events in somatic hybrid plants.

The fact that we were able to obtain a number of extremely asymmetric nuclear hybrids in this combination is not so surprising and probably due to an efficient selection. Indeed, it is well known from previous experiments that these species show a very high level of somatic incompatibility (Xianghui et al. 1982; Lavergne 1984; Pental et al. 1986). Moreover, *Nicotiana* cybrids with *Petunia* chloroplasts were easily obtained by Glimelius and Bonnett (1986). Therefore, we conclude that donor-recipient fusions, especially with species combinations that show a high level of somatic incompatibility, in association, with a good selection scheme are promising protocols for transferring small amounts of genetic material between plant species. Interesting addition or substitution lines might be created in this way which, due to their fertility, immediately could be integrated into a breeding scheme.

References

Agoudgil S, Hinnisdaels S, Mouras A, Negrutiu I, Jacobs M (1990) Metabolic complementation for a single gene function associated with partial and total loss of donor DNA in interspecific somatic hybrids. Theor Appl Genet 80:337–342

Bates GW, Hasenkampf CA, Contolini CL, Piastuch WC (1987) Asymmetric hybridization in *Nicotiana* by fusion of irradiated protoplasts. Theor Appl Genet 74:718–726

de Vries SE, Ferwerda MA, Loonen AEHM, Pijnacker LP, Feenstra WJ (1987) Chromosomes in somatic hybrids between *Nicotiana plumbaginifolia* and a monoploid potato. Theor Appl Genet 75:170–176

Dudits D, Fejér O, Hadlaczky G, Koncz C, Lazar GB, Horvath G (1980) Intergeneric gene transfer mediated by 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, Hinnisdaels S, Sidorov VA, Kaleda VA, Parokonny AS, Boryshuk NV, Cherep NN, Negrutiu I, Jacobs M (1988) Intergeneric asymmetric hybrids between *N. plumbaginifolia* and *Atropa belladonna* obtained by "gamma-fusion". Theor Appl Genet 76:760–766

Glimelius K, Bonnett HT (1986) *Nicotiana* cybrids with *Petunia* chloroplasts. Theor Appl Genet 72:794–798

Gupta PP, Gupta M, Schieder O (1982) Correction of nitrate reductase defect in auxotrophic plant cells through protoplast-mediated intergeneric gene transfers. 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

Han San L, Vedel F, Sihachakr D, Rémy R (1990) Morphological and molecular characterization of fertile tetraploid somatic hybrids produced by protoplast electrofusion and PEG-induced fusions between *Lycopersicon esculentum* Mill. and *Lycopersicon peruvianum* Mill. Mol Gen Genet 221:17–26

Harms CT (1983) Somatic incompatibility in the development of higher plant somatic hybrids. Q Rev Biol 58:325–351

Hinnisdaels S, Negrutiu I, Jcobs M, Sidorov VA (1988) Plant somatic cell hybridizations: evaluations and prospectives. IAPTC Newsl 55:2–10

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

Installé P, Negrutiu I, Jacobs M (1985) Protoplast-derived plants in *Nicotiana plumbaginifolia* viviana: improving the regenerating response of wild-type and mutant cultures. J Plant Physiol 119:443–454

Lavergne F (1984) Etude de la variabilité consécutive à la fusion somatique intergénérique entre *Petunia hybrida* et quelques Solanacées. Thèse de 3ème cycle, University Paris XI, France

Maniatis T, Fritsch EF, Sambrook J (1982) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press. Cold Spring Harbor, NY

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

Mouras A, Salesses G, Lutz A (1978) Sur l'utilisation des protoplastes en cytologie: amélioration d'une méthode récente en vue de l'indentification des chromosomes mitotiques des genres *Nicotiana* et *Prunus*. Caryologia 31:117–127

Mouras A, Wildenstein C, Salesses G (1986) Analysis of karyotype and c-banding pattern of *Nicotiana plumbaginifolia* using two techniques. Genetica 68:197–202

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15:473–497

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

Paszkowski J, Shillito RD, Saul M, Mandak V, Hohn T, Honh B, Potrykus I (1984) Direct gene transfer to plants. EMBO J 3:2717–2722

Pehu E, Karp A, Moore K, Steele S, Dunckley R, Jones MGK (1989) Molecular, cytogenetic and morphological characterization of somatic hybrids of dihaploid *Solanum tuberosum* and diploid *S. brevidens*. Theor Appl Genet 78:696–704

Pental D, Hamill JD, Pirrie A, Cocking EC (1986) Somatic hybridization of *Nicotiana tabacum* and *Petunia hybrida*. Recovery of plants with *P. hybrida* nuclear genome and *N. tabacum* chloroplast genome. Mol Gen Genet 202:342–347

Piastuch WC, Bates GW (1990) Chromosomal analysis of *Nicotiana* asymmetric somatic hybrids by dot blotting and in situ hybridization. Mol Gen Genet 222:97–103

Pijnacker LP, Ferwerda MA (1984) Giemsa c-banding of potato chromosomes. Can J Genet Cytol 26:415–419

Reiss B, Sprengel R, Will M, Schaller M (1984) A new sensitive method for qualitative and quantitative assay of neomycin phosphotransferase in crude cell extracts. Gene 30:211–218

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

Sjödin C, Glimelius K (1989a) *Brassica naponigra*, a somatic hybrid resistant to *Phoma lingam*. Theor Appl Genet 77:651–656

Sjödin C, Glimelius K (1989b) Transfer of resistance against *Phoma lingam* to *Brassica napus* by asymmetric somatic hybridization combined with toxin selection. Theor Appl Genet 78:513–520

Smith MA, Pay A, Dudits D (1989) Analysis of chloroplast and mitochondrial DNAs in asymmetric somatic hybrids between tobacco and carrot. Theor Appl Genet 77:641–644

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

Tanno-Suenaga L, Ichikawa H, Imamura J (1988) Transfer of the cms trait in *Daucus carota* L. by donor-recipient protoplast fusion. Theor Appl Genet 76:855–860

Wallroth M, Gerats AGM, Rogers SG, Fraley RT, Horsh RB (1986) Chromosomal localization of foreign genes in *Petunia hybrida*. Mol Gen Genet 202:6–15

Wijbrandi J, Zabel P, Koornneef M (1990) Restriction fragment length polymorphism analysis of somatic hybrids between *Lycopersicon esculentum* and irradiated *L. peruvianum:* Evidence for limited donor genome elimination and extensive chromosome rearrangements. Mol Gen Genet 222:270–277

Xianghui L, Wenbin L, Meijuan H (1982) Somatic hybrid plants from intergeneric fusion between tobacco tumor B6S3 and *Petunia hybrida* W43 and expression of LpDH. Sci Sin 25:611–619

Yamashita Y, Terada R, Nishibayashi S, Shimamoto K (1989) Asymmetric somatic hybrids of *Brassica:* partial transfer of *B. campestris* genome into *B. oleracea* by cell fusion. Theor Appl Genet 77:189–194

Yang ZQ, Shikanai T, Yamada Y (1988) Asymmetric hybridization between cytoplasmic male-sterile (CMS) and fertile rice (*Oryza sativa* L.) protoplasts. Theor Appl Genet 76:801–808