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Nuclear genomic composition of asymmetric fusion products between irradiated transgenic *Solanum brevidens* and *S. tuberosum*: limited elimination of donor chromosomes and polyploidization of the recipient genome

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Summary. The production of asymmetric somatic hybrid calli after fusion between gamma-irradiated protoplasts from transgenic *Solanum brevidens* and protoplasts from *S. tuberosum* are reported. Transgenic (kanamycin-resistant, GUS-positive) *S. brevidens* plants and hairy root clones were obtained after transformation with *Agrobacterium tumefaciens* LBA 1060 (pRi1855) (pBI121) and LBA 4404 (pRAL4404) (pBI121), and *A. rhizogenes* LBA 9402 (pRi1855) (pBI121), respectively. Leaf protoplasts isolated from the transgenic plants or root protoplasts from the hairy root clones were fused with *S. tuberosum* leaf protoplasts, and several calli were selected on kanamycin-containing medium. The relative nuclear DNA content of the hybrid calli was measured by flow cytometry (FCM), and the percentages of DNA of the *S. brevidens* and *S. tuberosum* genomes in the calli were determined by dot blot analysis using species-specific DNA probes. Chromosome-specific restriction fragment length polymorphism (RFLP) markers were used to investigate the elimination of specific *S. brevidens* chromosomes in the hybrids. The combined data on FCM, dot blot and RFLP analysis revealed that 18–62% of the *S. brevidens* DNA was eliminated in the hybrid calli and that the RFLP marker for chromosome 7 was absent in seven out of ten calli. The absence of RFLP markers for chromosomes 5 and 11 hardly ever occurred. In most of the hybrids the ploidy level of the *S. tuberosum* genome had increased considerably.

Key words: *Solanum brevidens* – *Solanum tuberosum* – *Agrobacterium* transformation – Asymmetric somatic hybrids – Restriction fragment length polymorphism (RFLP)

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Introduction

Somatic hybridization offers the possibility to transfer desirable traits across sexual borders or taxonomic distance. However, the fusion of protoplasts with whole genomes from two different plant species has often resulted in genetically complex hybrids with many unwanted characters. Asymmetric somatic hybridization using irradiated donor protoplasts is at present one of the approaches that is used for partial genome transfer. This method may also result in various types of fusion products or hybrid plants that cannot be predicted beforehand, e.g. cybrids (Sidorov et al. 1987; Kyozuka et al. 1989; Yarrow et al. 1990; Perl et al. 1990; Bonnema et al. 1991), hybrids with one or a few chromosomes (Dudits et al. 1987; Piastuch and Bates 1990) or hybrids with only a limited elimination of the donor chromosomes (Gleba et al. 1988; Sacristan et al. 1989; Yasmashita et al. 1989; Famelaer et al. 1989; Wijbrandi et al. 1990; Wolters et al. 1991). While the factors that induce the elimination of the donor chromosomes and determine the ultimate genome composition of the hybrid regenerants are not exactly known, the irradiation dose seems to be of minor importance (Famelaer et al. 1989; Wijbrandi et al. 1990), whereas the taxonomic distance between the fusion parents my play an important role (Derks et al. 1992).

In potato, asymmetric hybridization between *Solanum tuberosum* and *S. brevidens* is of considerable interest, as the latter species constitutes a rich source of many agronomically important traits, such as resistance to potato leaf roll virus and *Erwinia* soft rot (Helgeson et al. 1986). This article presents data on the production and analysis of asymmetric hybrid calli obtained after protoplast fusion between gamma-irradiated *S. brevidens* and a diploid *S. tuberosum*. Protoplasts isolated from transgenic plants or hairy root clones of *S. brevidens*

carrying kanamycin resistance were used for the fusions. Putative hybrid calli were selected on kanamycin-containing medium, and genome composition was determined by means of chromosome-specific restriction fragment length polymorphism (RFLP) markers and dot blotting.

Materials and methods

Genotypes

In vitro shoot cultures of two diploid *S. brevidens* (2n = 2 × = 24) clones – PI 218228, obtained from J.P. Helgeson, Madison, Wis., USA, and PI 245763, obtained from the Department of Plant Breeding, Agricultural University, Wageningen, the Netherlands – and two *S. tuberosum* clones (2n = 2 × = 24) – the dihaploid DH 81-7-1463 and the diploid SH 81-128-1579 – maintained on Murashige and Skoog (MS) medium with 3% sucrose (MS30) were used in the present experiments.

Transformation of S. brevidens

Agrobacterium tumefaciens strains LBA 1060 (pRi1855) (pBI121) or LBA 4404 (pRAL4404) (pBI121) and *A. rhizogenes* strain LBA 9402 (pRi1855) (pBI121) were used for transformation of *S. brevidens*. The plasmid pRi1855 contains TR-DNA as well as TL-DNA with the *rol* genes. Expression of these genes leads to the production of hairy roots (Vilaine and Casse-Delbart 1987). The plasmid pRAL4404 is a helper plasmid carrying the vir-region (Hoekema et al. 1983). The plasmid pBI121, a derivative of Bin 19 (Bevan 1984), contains the *NPTII* gene with NOS promoter and terminator and the β-glucuronidase (GUS) gene with CaMV35SRNA promoter and NOS terminator.

Leaf and stem segments were infected with a LBA 1060 or LBA 9402 *Agrobacterium* suspension for 15 min and then transferred to H_2O-agar after washing for co-cultivation for two days. The infected segments were subsequently transferred to selection medium MS30 supplemented with 350 mg/l cefotaxime and 100 mg/l kanamycin. Hairy roots developed during the 1–3 weeks of culture on this medium. Eventually, the hairy roots were removed and separately cultured as root clones on the selection medium. Callus was induced only on segments of the hairy roots obtained after infection with LBA 1060 and using MS medium supplemented with 2% sucrose (MS20) and 0.05 mg/l 2,4 dichlorophenoxyacetic acid (2.4 D), 1.0 mg/l zeatine and 350 mg/l cefotaxime. Shoots were regenerated from the calli cultured on MS medium supplemented with 1 or 3% sucrose (MS10 or MS30) with 1.0 mg/l zeatine and 2 mg/l gibberellic acid (GA3). The shoots that were regenerated after 2–3 months were able to develop further and form roots when grown in liquid MS30 supplemented with 350 mg/l cefotaxime in 100 ml flasks on a rotary shaker for 2–4 weeks (Ottaviani et al. 1990). Plantlets were transferred to MS30 for further growth.

Leaf and stem segments were also infected with another *A. tumefaciens* strain, LBA 4404. Infected segments were co-cultivated on MS30 with 1.0 mg/l indoleacetic acid (IAA) and 2.5 mg/l benzylaminopurine (BAP) for 2 days. Selection was carried out on the same medium after addition of 100 mg/l kanamycin and 350 mg/l cefotaxime. Also, in one experiment, *Petunia* feeder cells were used prior to infection of the leaf segments as well as during co-cultivation. After 4 weeks, adventitious shoots appeared without any visible callus phase. These 0.5- to 1.0-cm-long putative transgenic shoots were transferred first to liquid MS30 for rooting and further growth and subsequently to solid MS30.

GUS activity (X-Gluc) tests (Jefferson et al. 1987) and Southern blot hybridization were carried out to confirm the transformation events and determine the number of insertions, respectively (see Southern blot hybridization).

Protoplast fusion

Protoplasts of *S. brevidens* were irradiated in the enzyme mixture with a lethal dose of 0.5 kGy Cobalt 60 gamma rays (dose rate 25–30 Gy/min) and then electrofused with fluorescein diacetate (FDA, 3 mg/l)-stained protoplasts isolated from bleached *S. tuberosum* plantlets (Mattheij and Puite 1992). Also, in a number of experiments FDA-stained protoplasts isolated from *S. brevidens* hairy root clones were electrofused with *S. tuberosum* mesophyll protoplasts. For isolation of the root protoplasts, 1- to 2-cm-long root segments were cultured in liquid MS20 supplemented with 0.05 mg/l BAP and 0.3 mg/l NAA to induce lateral roots (Laine and Ducreux 1987). After 1 week, the root segments were cut into small pieces 1–2 mm in length, and the medium was replaced by the enzyme mixture (1% cellulase R10, 0.1% pectolyase Y-23 in half strength V-KM with 0.2 *M* mannitol and 0.2 *M* glucose, pH 5.6). The protoplast yield was $2-6 \times 10^5$ per gram wet root material after an overnight incubation.

Electrofusion and culture were carried out following procedures described earlier (Puite et al. 1986). Putative hybrid cell colonies were selected by adding 50 mg/l kanamycin (final concentration) to the culture medium upon dilution 1–2 weeks after fusion. Putative hybrid calli were selected on solid medium [MS10 with 1 mg/l BAP and 0.2 mg/l naphtalene acetic acid (NAA)] supplemented with 100 mg/l kanamycin to which the calli were transferred 6 weeks after fusion. Actively growing calli were transferred to shoot induction medium MS10 supplemented with 1 mg/l zeatine and 0.01 mg/l NAA.

Determination of nuclear DNA content

The nuclear DNA content of the transgenic plants and calli was measured by means of an Ortho ICP 22 flow cytometer using a lysis buffer (De Laat and Blaas 1984) supplemented with 2 µl/ml 4,6 diamidino-2-phenylindole (DAPI). Fixed calf thymocytes or chicken erythrocytes were used as the standard, and mesophyll cells from *S. brevidens* and *S. tuberosum* were included for comparison.

Southern blot hybridization

DNA isolation from leaf material was carried out according to Bonierbale et al. (1988). For the isolation of DNA from callus tissue, the procedure described by Mettler et al. (1987) was followed with the addition of a phenol/chloroform and chloroform purification step. Polysaccharides were precipitated with 0.1 volume 96% ethanol (5 min at 0 °C), and protein precipitation was realized by the addition of 0.25 volume 5 *M* KAc to the supernatant followed by incubation for 20 min at 0 °C. About 5 µg DNA of each sample was digested with *Hin*dIII or *Eco*RI. As a routine procedure gelatine (0.01%) and spermidine (2 m*M*) were added to the restriction enzyme mixture to improve digestion. After electrophoresis in 0.7% agarose gels DNA fragments were transferred to a Hybond-N nylon membrane. The transformation of *S. brevidens* was confirmed by hybridization with the 1.5-kbp NPTII gene as a *Hin*dIII-*Sal*I fragment in pACYC184 and the 2.0-kbp GUS gene with the terminator from pBI101.1 (Jefferson et al. 1987) as a *Hin*dIII-*Eco*RI fragment in pUC19 used as probes.

RFLP analysis

For the RFLP analysis, TG probes and a CD probe were used (Bernatzky and Tanksley 1986; Bonierbale et al. 1988). The choice of the RFLP probes was based on the map of RFLP

markers for *S. tuberosum* and *S. brevidens* reported by Williams et al. (1990). Probes were labeled with digoxigenine-dUTP and used for DNA-DNA hybridization according to the procedure of Kreike et al. (1990) with a washing buffer containing 0.5% SDS instead of 0.1% SDS. The blots with parental and hybrid DNA could be used about 10 times after stripping.

Dot blot analysis

Dot blot analysis was carried out according to Derks et al. (1992). DNA of the parents in a series of 0–400 ng DNA/dot and DNA of the hybrids (200 ng/dot, three or four replicates) were transferred to a Gene Screen Plus membrane (Du Pont) using a Hybrid Dot 96-well manifold (Gibco/BRL). Two identical dot blots were prepared. Hybridizations were carried out with two species-specific highly repetitive DNA probes, i.e. the high copy number *S. tuberosum*-specific probe pST10 and the *S. brevidens*-specific probe pSB7. Both clones are likely to be dispersed repeats and were a generous gift from Dr. E. Pehu, Helsinki (Pehu et al. 1990). The probes were labeled with [α-^{32}P]dATP. After exposure to a X-ray film (Kodak X-Omatic AR), the dots were cut from the membrane and radioactivity was measured with a Tri-carb liquid scintillation analyser. Data were corrected for background.

Results

Transformation and regeneration

Transformation data are shown in Table 1. In all, 64 hairy root clones were selected after transformation with the LBA 1060 and LBP 9402 strains. Three plants were regenerated from the 8 root clones derived after transformation with LBA 1060. Of these plants, one was diploid (1-1A) and two were tetraploids (11-1C and 13-1A) as measured by flow cytometry (FCM). The plants exhibited the typical hairy root phenotype (i.e. crinkled leaves and abundant rooting) and were GUS positive (Table 2). Five of the root clones derived after the transformation of *S. brevidens* PI 245763 with LBA 9402 and showing fast growth on the selective medium were also tested for GUS expression. Four clones were GUS positive and diploid (Table 2). Of the 19 plants obtained after transformation of PI 218228 with LBA 4404, 16 were tested for GUS expression. Thirteen plants were GUS positive, including KG5, KG6 and KG10, which were all diploids.

Southern blot hybridization

Plantlets were transferred to the greenhouse and analysed after 2½ months for stable transformation by Southern blot hybridization using the *NPTII* and GUS probes with *Hin*dIII as the restriction enzyme (Fig. 1). *Hin*dIII was used because a single *Hin*dIII site is located between the NPTII and GUS gene in the plasmid. The number of *NPTII* and GUS bands reflecting the number of insertions differed in some plants (Table 2), while the intensity of the bands in the same lane also showed differences. The latter may indicate that fragments of about the same length were present. No hybridization could be observed for the KG5 plants. Apparently, the KG5 mother plant was not stably transformed and had lost the introduced genes. However, it is of interest that hybrid calli nos. 61 and 227, which were obtained from fusions with 3-month-old in vitro-grown KG5 plants, did contain the *NPTII* gene, as confirmed by Southern blot hybridization.

Table 2. GUS activity of in vitro shoot cultures and hairy root clones, and the data on Southern blot hybridization of DNA from greenhouse-grown plants with the *NPTII* and GUS probes

S. brevidens plants and root clones	GUS activity of in vitro shoot cultures and root clones	Number of bands in greenhouse-grown plants	
		NPTII	GUS
PI 245763 (wt)	–	0	0
1.1 A	+	1	1
11.1 C	+	2	4
13.1 A	+	3	3
4 root clones	+	nr	nr
1 root clone	–	nr	nr
PI 218228 (wt)	–	0	0
KG5	+	0	0
KG6	++	2	1
KG10	++	1	2

–, No GUS activity; + and ++, moderate and high GUS activity, respectively; nr, not relevant

Table 1. Transformation of *Solanum brevidens* clones by various strains of *Agrobacterium*

Bacterial strains	*S. brevidens* clones	Number of experiments	Explant source	Number of explants	Root clones obtained	Regenerated plants
LBA 1060	PI 245763	1	Leaf	47	8	3[a]
			Stem	5	0	–
LBA 9402	PI 245763	2	Leaf	113	14	–
			Stem	125	33	–
LBA 9402	PI 218228	4	Leaf	276	4	–
			Stem	242	5	–
LBA 4404	PI 218228	2	Leaf	350	–	19[b]

[a] Plants designated 1-1A, 11-1C and 13-1A
[b] Three of these plants are designated KG5, KG6 and KG10

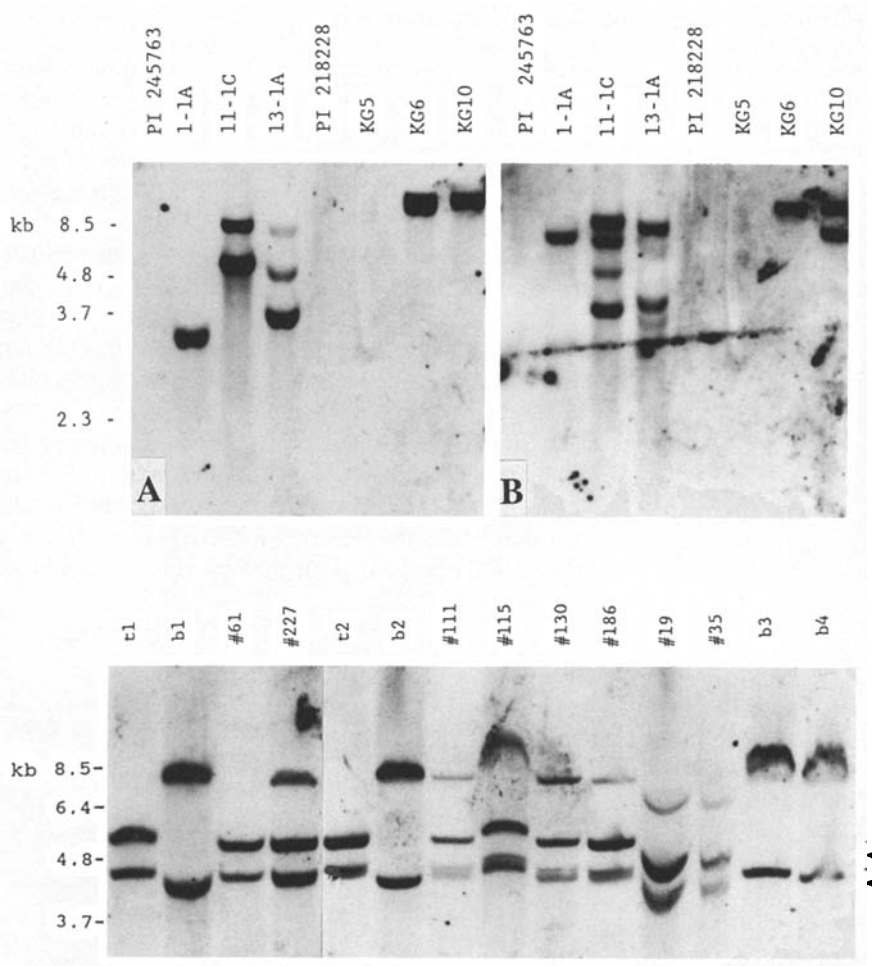

Fig. 1 A, B. Southern blot hybridization of plants regenerated after transformation of *S. brevidens* with the *Agrobacterium tumefaciens* strains LBA 1060 or LBA 4404. DNA was digested with the restriction enzyme *Hin*dIII and hybridized with a 1.5-kbp *NPTII* gene fragment (**A**) or a 2-kbp GUS + terminator fragment (**B**) that were used as a probes

Fig. 2. RFLP analysis of eight hybrid calli obtained after fusion between irradiated transgenic *S. brevidens* protoplasts and non-irradiated protoplasts of *S. tuberosum*. ⬅ A *S. tuberosum*-specific band; ⇦ a *S. brevidens*-specific band. The *lanes b* and *t* correspond to *S. brevidens* and *S. tuberosum* parents, respectively. The fusion combination b1(+)t1 resulted in the hybrid calli nos. *61* and *227*; b2(+)t2 in nos. *111, 115, 130* and *186*; b3(+)t2 in no. *19*; b4(+)t2 in no. *35* (*b1* KG5, *b2* KG10, *b3* 11.1C, *b4* 13.1A, *t1* DH 81-7-1463, *t2* SH 81-128-1579). Hybridization was carried out with the probe-enzyme combination TG35/*Hin*dIII, giving RFLP for chromosome 9

Protoplast fusion and nuclear DNA content of putative hybrid calli

Table 3 presents data on the number of kanamycin-resistant calli that developed after fusion between irradiated transgenic *S. brevidens* protoplasts and non-irradiated *S. tuberosum* protoplasts and their nuclear DNA content. Unfused parental protoplast mixtures did not develop further to proliferating calli on kanamycin-containing medium. The nuclear G1 DNA content of *S. brevidens* and the calli was measured by FCM and estimated relative to the nuclear G1 DNA content of *S. tuberosum*, which was taken to be 2.0. The relative DNA content was estimated to be 1.7 and 3.4 for the diploid and tetraploid *S. brevidens* parents, respectively. The data show that the calli exhibited a wide range of DNA content per nucleus with increased DNA levels. A number of actively growing calli were transferred to the regeneration medium, but no shoots could be induced.

RFLP analysis

Eleven hybrid calli obtained after fusion between irradiated transgenic *S. brevidens* protoplasts and non-irradiated *S. tuberosum* protoplasts, especially those calli with a low DNA content, were analysed with regard to their chromosome composition using RFLP markers. Data on the presence or absence of the *S. brevidens* chromosome-specific markers in the hybrids are given in Table 4. A representative example of the analysis of 8 calli is shown in Fig. 2.

DNA was digested with *Hin*dIII only because of the low yield of DNA isolated from the slow-growing calli.

Table 3. Frequency distribution of putative hybrid calli selected on kanamycin-containing medium after fusion between protoplasts of irradiated transgenic *S. brevidens* and non-irradiated *S. tuberosum* with respect to their relative nuclear DNA content

Donor plants	Ploidy level	Number of calli obtained	Frequency distribution of the relative nuclear DNA content of some of the calli				Identification numbers of the calli further analysed
			0–4.0	4.1–6.0	6.1–10	>10	
1-1 A	2×	20	0	4	4	3	3, 5
11-1 C	4×	7	0	1	1	1	19
13-1 A	4×	13	0	1	4	5	35, 38
Root clones	2×	15	0	5	6	0	
KG5	2×	26	2	8	6	10	60, 61, 62, 227
KG6	2×	4	0	1	0	3	
KG10	2×	109	1	20	47	38	111, 115, 130, 144, 159, 186

Table 4. RFLP analysis of the hybrid calli obtained after fusion between irradiated transgenic *S. brevidens* protoplasts and non-irradiated *S. tuberosum* protoplasts

S. brevidens chromosome and DNA probe	Donor[a]	1.1A	1.1A	11.1C	13.1A	13.1A	KG5	KG10	KG10	KG10	KG10	KG5
	Ploidy	2×	2×	4×	4×	4×	2×	2×	2×	2×	2×	2×
	Hybrid	3	5	19	35	38	61	111	115	130	186	227
	DNA[b]	4.7	4.9	4.9	5.2	6.1	3.9	7.7	11.6	9.7	4.4	4.5
1, –		ni[c]	ni	ni	ni	ni	ni	ni	ni	ni	ni	ni
2, TG48		+	ni	–	–	–	–	+	+	+	+	–
3, TG56		+	±	+	+	ni	–	–	+	+	+	+
4, TG22		+	ni	+	ni	–	–	+	+	+	–	+
5, TG32		+	+	+	+	+	±	+	+	+	–	+
6, TG115		+	ni	–	+	–	–	+	+	+	+	+
7, TG61		–	ni	+	–	+	–	–	+	–	–	–
8, TG176		ni	ni	ni	ni	ni	–	ni	ni	ni	ni	–
9, TG35		–	–	+	+	–	–	+	+	+	+	+
10, TG63		–	ni	+	+	+	–	–	+	+	+	–
11, TG36		+	ni	±	±	±	±	±	±	±	–	±
12, CD22		ni	ni	ni	ni	ni	–	ni	ni	ni	ni	–

[a] Transgenic *S. brevidens* plants used for protoplast isolation and fusion
[b] Relative nuclear DNA content of the hybrid calli
[c] + and –, Presence and absence, respectively, of chromosome-specific bands; ±, weak bands; ni, no information is available

Of the many probes tested, 9 gave clear RFLPs between *S. brevidens* and *S. tuberosum* with similar hybridization patterns for TG22, TG35, TG36, TG61, TG63 and TG115 as reported by Williams et al. (1990). However, in contrast with the results of these authors, probe TG24 (chromosome 1) did not give a useful RFLP, probably due to the different *S. tuberosum* genotype used. For chromosomes 1, 8 and 12, a number of probe/*Hin*dIII combinations were tested. However, no suitable RFLP for chromosome 1 could be found, whereas hybridization with probes TG176 and CD22 gave a RFLP for chromosomes 8 and 12, respectively, although the signal was weak due do the repeated use of the blot. The *S. tuberosum*-specific markers were present in all of the hybrids. At least 9 *S. brevidens*-specific RFLP markers were absent in hybrid no. 61, while the other hybrids had lower numbers of absent bands (Table 4).

Dot blot analysis

The amount of DNA from the donor and the recipient parents that was present in the hybrid calli was determined with the species-specific repetitive DNA probes pST10 and pSB7. Linear regression curves were established for each parent on the basis of the autoradiograms of the dot blots (Fig. 3). The angles of declination of the curves of the two *S. tuberosum* parents (DH 81-7-1463 and SH 81-128-1579) and also of the four *S. brevidens* parents (1.1A, 11.1C, 13.1A, KG5, KG6 and KG10) were within statistical error (results not shown in Fig. 3). Consequently, from these two sets of curves a weighted mean calibration curve for *S. tuberosum* and for *S. brevidens* was determined, and the amount of DNA from each parent present in the hybrids was calculated on the basis of the nuclear DNA content measured by FCM.

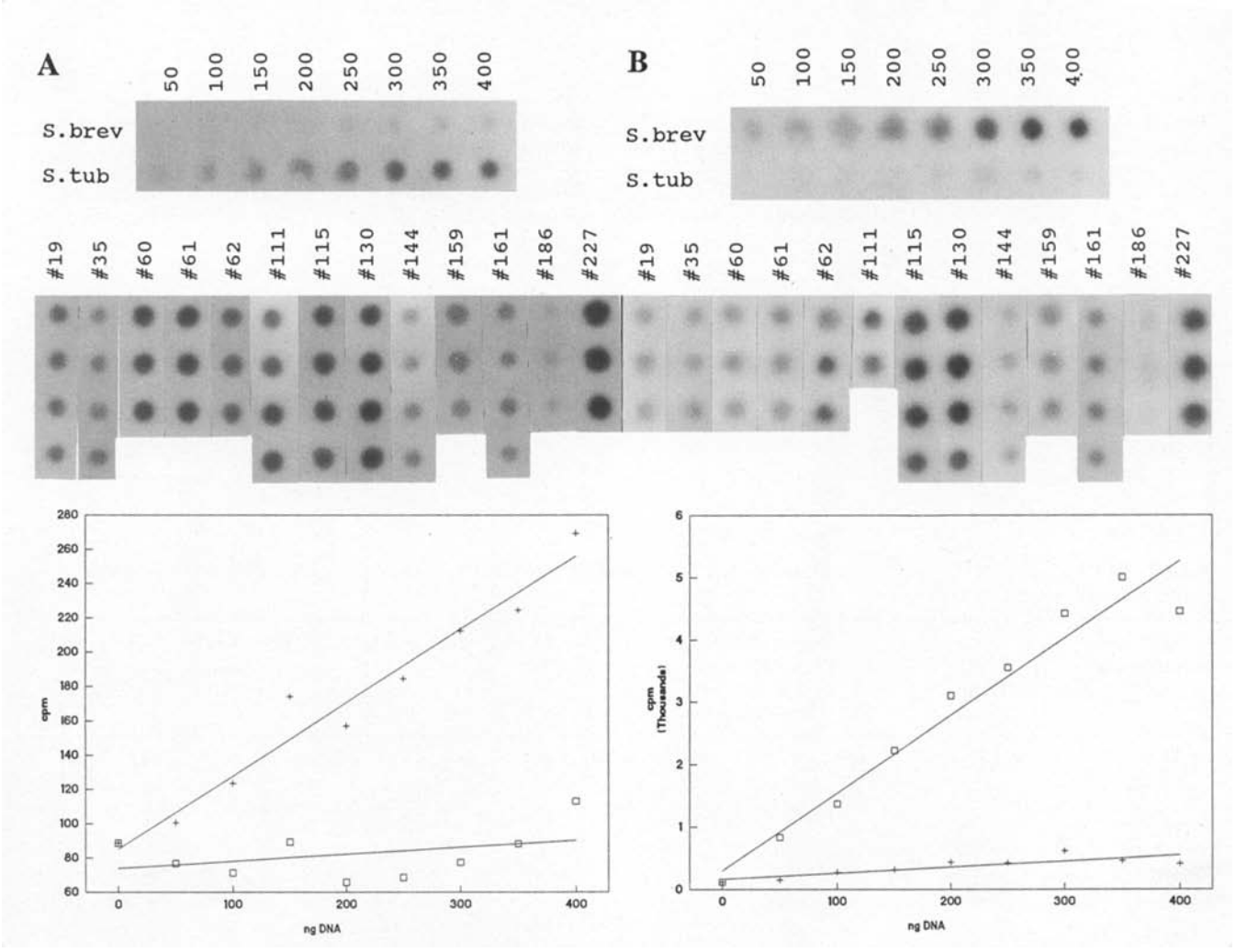

Fig. 3A, B. Dot blot analysis. A concentration series (0–400 ng DNA) of *S. tuberosum* and *S. brevidens* parental DNA and DNA from the hybrid calli (200 ng) was spotted on two blots and hybridized with the *S. tuberosum*-specific probe pST10 (**blot A**) and with the *S. brevidens*-specific probe pSB7 (**blot B**). Calibration curves were obtained via linear regression for each of the parents and for both probes. A DNA concentration series and the calibration curves of *S. tuberosum* SH 81-128-1579 (+) and *S. brevidens* KG10 (□) are shown

Table 5. Flow cytometric determination of relative nuclear DNA content and dot blot analysis using species-specific DNA probes: columns 3 and 5, parental nuclear DNA content in the hybrids, calculated from the percentage of parental DNA in the hybrids as determined by dot blotting; columns 4 and 6, estimated ploidy levels of the parental genomes in the hybrids

Parents and hybrid calli	Relative nuclear DNA content	Relative DNA content *S. tuberosum* genome	Estimated ploidy level of *S. tuberosum* genome	Relative DNA content *S. brevidens* genome	Estimated ploidy level of *S. brevidens* genome
S. tuberosum	2.0	2.0	2×		
S. brevidens	1.7; 3.4			1.7; 3.4	2×, 4×
19[a]	4.9	3.6	4×	1.3	2×
35[a]	5.2	3.7	4×	1.5	2×
60	3.9	3.1	3×	0.8	1×
61	3.9	3.2	3×	0.7	1×
62	13.0	8.5	8×	4.5	5×
111	7.7	5.5	6×	2.2	3×
115	11.6	5.6	6×	6.0	7×
130	9.7	5.2	5×	4.5	5×
144	8.3	7.0	7×	1.3	2×
159	5.4	3.6	4×	1.8	2×
161	4.3	2.2	2×	2.1	2×
186	4.4	3.7	4×	0.7	1×
227	4.5	3.1	3×	1.4	2×

[a] Tetraploid *S. brevidens* was used for fusion

Table 5 shows the data obtained from the FCM histograms and the dot blot analysis of 13 hybrid calli. From these, the ploidy levels of the *S. tuberosum* and *S. brevidens* genomes present in the hybrids were estimated. Hybrids nos. 60, 61 and 186, and also 19 and 35 contained substantially less *S. brevidens* nuclear DNA than their *S. brevidens* parents.

Discussion and conclusions

The hybrid calli obtained after fusion between gamma-irradiated transgenic *S. brevidens* protoplasts and non-irradiated *S. tuberosum* protoplasts contained a greater amount of DNA per nucleus than the sum of the DNA content of the two parents, except for the hybrid calli nos. 19 and 35, both of which originated from fusions between the tetraploid *S. brevidens* donor and the *S. tuberosum* recipient. In most of the hybrids the ploidy level of the recipient parental genome increased considerably. Polyploidization of the recipient genome after asymmetric fusion has also been observed by others (Gleba et al. 1988; Wijbrandi et al. 1990). Strikingly, the increase in the ploidy level of the donor occurred only in hybrids that contained a higher nuclear DNA content. Elimination of the *S. brevidens* nuclear DNA ranged from 18% to 62% in various hybrid calli. The percentage of donor DNA in the hybrid calli was dependent on the amount of recipient DNA: the level of donor DNA was the highest when there was a large amount of DNA of the recipient genome. This suggests that polyploidization of the recipient *S. tuberosum* genome may be a necessary mechanism by which to obtain the proliferation at high numbers of the irradiated damaged donor *S. brevidens* chromosomes.

Limited donor (*S. tuberosum*) DNA elimination has also been observed in hybrid calli derived from fusions between irradiated *S. tuberosum* protoplasts and *Lycopersicon esculentum* protoplasts (Wolters et al. 1991). In this case, selection was based on a cytoplasmically controlled chlorophyll deficiency of the recipient. A possible explanation for the limited elimination of DNA suggested by the authors was that nuclear DNA of the donor may be required for the donor chloroplasts to be functional. In fusions between gamma-irradiated (0.1 kGy) *S. brevidens* protoplasts and iodoacetate-treated *S. tuberosum* protoplasts albino-like regenerants, possibly cybrids, were obtained in which the chloroplast DNA was derived from the donor (Perl et al. 1990). However, in the present study, one or a few chromosomes carrying the NPTII gene(s) would always be present in the fusion product because the selection was based on kanamycin resistance (a nuclear trait). The presence of several more chromosomes in the hybrid calli relative to the number of NPTII gene insertions may indicate that the presence of certain chromosomes is important for callus proliferation. For example, chromosomes 5 and 11 were present in almost all of the hybrid calli, as ascertained by chromosome-specific RFLP markers, and these may carry essential information for cell proliferation. The elimination of certain specific chromosomes in all of the hybrid calli was not observed, although the marker for chromosome 7 was absent in seven out of ten calli. The complex genome constitution of the hybrid calli may be the reason for the the lack of shoot regeneration.

It is of interest to compare RFLP and dot blot data, considering that the presence or absence of a RFLP marker may not necessarily imply the presence or absence of a certain intact chromosome (Table 4 and 5). Assuming a complete elimination of all homologous chromosomes in the case of the absence of a RFLP band, one may conclude that the RFLP and dot blot data support each other and are not conflicting. Comparison of the data suggests that in the hybrid calli nos. 19 and 35, some of the *S. brevidens* chromosomes for which no RFLP information could be collected (chromosomes 1, 8 and 12) are possibly absent.

It is clear that asymmetric hybrids with only a few *S. brevidens* chromosomes could not be obtained, even when a high radiation dose was applied. As a diploid potato has a high tendency to become tetraploid in protoplast culture (Ramulu et al. 1989), the use of tetraploid instead of diploid *S. tuberosum* as a recipient may possibly result in more stable and less complex hybrid genomes without the loss of regeneration capacity.

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