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Asymmetric somatic hybridization between tomato (*Lycopersicon esculentum* Mill) and gamma-irradiated potato (*Solanum tuberosum* L.): a quantitative analysis

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Abstract. We analyzed 110 asymmetric fusion products, obtained by fusion of hygromycin-resistant tomato protoplasts and gamma-irradiated kanamycin-resistant potato protoplasts that expressed β-glucuronidase (GUS). The fusion products were selected for resistance to both antibiotics, and were subsequently analyzed for their shoot regeneration potential, GUS activity, expression of two potato isoenzymes, chloroplast type, total genomic DNA content, and relative genomic composition. No viable plants could be obtained and the calli were highly polypoid. All hybrids expressed GUS activity, whereas they displayed a large variation with respect to the other traits.

Key words: Tomato – Potato – Asymmetric somatic hybridization – DNA elimination – Genome and plastome constitution

Introduction

Asymmetric somatic hybridization is often described as a new plant breeding technique that allows the introduction of genes from a donor species into a recipient crop species, and can be useful if these species cannot be hybridized sexually. Asymmetric hybridization can be performed by fusion of protoplasts of a recipient species with protoplasts of a donor species of which the nuclear DNA has been damaged e.g., by ionizing irradiation. Ideally, the resulting asymmetric somatic hybrids combine the complete recipient genome with a small fraction of the donor genome. Further elimination of unfavourable, and the preservation of favourable, donor traits can be achieved by means of recurrent sexual hybridization with the recipient species and selection for the desired traits. In some cases, asymmetric somatic hybrids have been successfully used as parents in subsequent sexual hybridization (Dudits et al. 1980, 1987; Somers et al. 1986; Gleba et al. 1988; Sjödin and Glimelius 1989; Bates 1990). Most of these partially-fertile hybrids are highly asymmetric: they contain only a minor part of the donor genome. However, many reports describe poor growth and regeneration of asymmetric fusion products, limited elimination of donor DNA, high aneuploidy, strong polyploidization and, in cases where plants were obtained, sterility of the asymmetric somatic hybrids (e.g. Itoh and Futsuhara 1983; Imamura et al. 1987; Sacristan et al. 1989; Yamashita et al. 1989; Wijbrandi et al. 1990a, b; Hinnisdaels et al. 1991; Wolters et al. 1991).

Little information is available about the factors that influence regeneration, chromosome elimination, and polyploidization, and the relations between these various biological processes after asymmetric hybridization. For example, one can envisage that there may be a selective advantage in the case of asymmetric somatic hybrids with higher ploidy levels, and that this advantage subsequently affects shoot regeneration in a negative way. In addition, the role of organelle DNA should be considered because cybrids with potato chloroplast DNA but without potato nuclear DNA were not obtained after fusion of tomato protoplasts with gamma-irradiated potato protoplasts (Wolters et al. 1991). The incongruity between a recipient nucleus and donor chloroplasts, associated with large phylogenetic distances between parental plants, was also reported by Derks et al. (1992) and Wolters et al. (1993) for combinations of other solanaceous species.

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The present study describes the genetic characterization of a large number of asymmetric fusion products that resulted from fusion of protoplasts from two hygromycin-resistant tomato genotypes with gamma-irradiated protoplasts from a kanamycin-resistant, β-glucuronidase (GUS)-expressing potato genotype. Fusion products were obtained by selection for resistance against both antibiotics and then analyzed with respect to several other unselected parental traits. The chromosomal complement of these fusion products was also analyzed.

Materials and methods

Plant material

The tomato recipient genotype was obtained by crossing genotype *Xa-2/xa-2* (Persson 1960) with MsK9 (Koornneef et al. 1987a). From the F_1 progeny an *Xa-2/xa*-genotype, designated Xa11, with good regeneration capacity from roots and hypocotyl, was selected according to Koornneef et al. (1993). Transformation of Xa11 with *Agrobacterium tumefaciens* strain Ach5, containing the plasmid pJW6 which transfers hygromycin-B resistance, was carried out as described by Koornneef et al. (1987b). Two diploid, hygromycin-resistant transformants, designated Xa11-H4 and Xa11-H8, which did not show any other visually-disturbed phenotype, were used in protoplast fusion experiments. For potato the monoploid (2n=x=12) genotype 7322, which originates from Prof. Dr. G. Wenzel, Germany (for detailed description see de Vries et al. 1987), was transformed in the same way with *A. tumefaciens* strain C58, containing the plasmid pZ707C which transfers kanamycin resistance and the β-glucuronidase (GUS) reporter gene (Jefferson et al. 1987). A somatically-doubled diploid (2n=x=24) kanamycin-resistant GUS-expressing transformant, designated 7322-K5, was used as 'donor' genotype in protoplast fusion experiments.

Protoplast isolation, gamma irradiation, electrofusion and culture; selection of fusion products and regeneration

Protoplast isolation, gamma irradiation, electrofusion and culture were carried out as described by Wolters et al. (1991). Fusion products were selected on culture media containing 50 mg/l of hygromycin-B and 150 mg/l of kanamycin, from 1 week until half a year after fusion. Minicalli were transferred to solidified TMCμZ greening medium (Wolters et al. 1991) 1 month after fusion and subcultured every month. Larger calli were transferred to 1Z medium (Wijbrandi et al. 1990b), approximately 3 to 6 months after fusion, for shoot regerenation. Calli were classified as regenerable when small shoot-like structures were observed. Regenerated shoots were grown on solidified medium according to Murashige and Skoog (1962) without hormones; 20 g/l of sucrose and 8 g/l of agar were added. The pH was adjusted to 5.8 before autoclaving. This medium was designated MS20.

Flurometric β-glucuronidase assay

β-Glucuronidase (GUS) activity was analysed according to Jefferson et al. (1987). Approximately 20 μg of callus tissue was ground in 20 μl of extraction and staining buffer containing 1 mM of 4-methyl umbelliferyl glucuronide (MUG; Sigma M-9130) as a fluorogenic substrate. After 15 min incubation at 37 °C the reaction was stopped with 0.2 ml of 1 M Na_2CO_3 and fluorescence was registered under UV light.

Flow cytometric analysis

For flow cytometric analysis of callus tissue approximately 0.5 g of tissue was placed in a 6-cm Petri dish and 1 ml of a nuclei buffer, consisting of 10 mM spermine tetrahydrochloride, 200 mM hexylene glycol, 10 mM NaCl, 10 mM Tris-HCl, 0.025% (v/v) triton-X100 and 2.5 μg/ml 4',6-diamidino-2-phenylindole (DAPI), pH 7.0, was added. The tissue was finely cut up with a sharp razor blade and the mixture was filtered through an 85-μm-pore nylon filter. The filtrate was directly used for analysis in a flow cytometer (IPC22, Ortho Diagnositic Systems, Beerse, Belgium). Calf thymocytes and chicken erythrocytes were used as internal, absolute standards; tomato haploids, diploids and tetraploids were used as references. The nuclear DNA content was expressed as a relative C-value.

In our flow cytometric analysis the diploid nuclear weights and relative C-values of tomato and potato were almost identical. Similar weights were also reported by Arumuganathan and Earle (1991).

DNA isolation, DNA probes, Southern-blot and dot-blot analysis

Total DNA from leaves of tomato MsK9 and potato 7322 and from fusion calli was isolated as described by Wolters et al. (1991). Probes were radioactively labelled by means of the Boehringer Mannheim Random Primed DNA Labelling Kit. The chloroplast DNA composition of fusion products was analyzed by hybridization of Southern blots of *Hin*fI-digested total DNA to the *Petunia hybrida* chloroplast DNA clone pPCY64 (Derks et al. 1991).

Determination of the ratio of tomato:potato genomic DNA was carried out by means of dot-blot analysis according to Wolters et al. (1991). Several concentrations of parental DNA were applied to two identical dot blots to make a calibration plot of the radioactivity per dot in relation to the amount of DNA from one species. Total DNA from the fusion products was applied to the same dot blots. Two identical filters were prepared for every analysis. One was probed with pTHG2, a tomato-specific repetitive DNA probe (Zabel et al. 1985). The insert of pTHG2 represents a moderately-repetitive DNA fragment that is evenly dispersed on all tomato chromosomes, as was shown by in-situ hybridization (Zabel et al. 1985). The other filter was probed with P5L, a potato-specific repetitive DNA probe (Visser et al. 1988). On all potato chromosomes hybridization with this fragment occurs predominantly in the telomeric and centromeric regions. With the calibration plots for both species-specific probes the amount of tomato and potato DNA per dot could be estimated, and the percentage of nuclear DNA of the fusion products that originated from potato could be determined.

The diploid nuclear weights of tomato and potato were considered identical. The flow cytometric data together with the dot-blot data were used to estimate the number of genome equivalents of tomato, potato, respectively that were present in hybrid calli.

Isoenzyme analysis

Glutamate oxaloacetate transaminase (GOT; EC 2.6.1.1) and malate dehydrogenase (MDH; EC 1.1.1.37) are dimeric enzymes. GOT and MDH zymograms of symmetric fusion products of tomato and potato not only display parental, homodimeric

bands but also intermediate, heterodimeric hybrid bands. The elimination of all potato alleles that encode for a specific isoenzyme subunit results in the disappearance of the corresponding heterodimeric, hybrid band in the zymogram.

Isoenzyme analysis of GOT and MDH was performed as follows. Approximately 0.25 g of fresh callus tissue was ground in 0.25 ml of 0.05 M Tris-HCl, 0.1 g/l bromophenol blue, 20% (v/v) glycerol and 1% (v/v) β-mercaptoethanol solution, pH 6.8. After centrifugation in an Eppendorf centrifuge for 5 min at maximum speed the supernatants were subjected to polyacrylamide-gel electrophoresis (PAGE) according to Schoenmakers et al. (1992). Staining reactions were performed according to Vallejos (1983).

Results

Selection of fusion products and regeneration

Tomato protoplasts were able to divide and regenerate plants in culture media lacking kanamycin. Without irradiation, potato microcalli could rarely be obtained in hygromycin-free media. These microcalli turned brown and died at a very early stage. After gamma irradiation with a dose of 150 or 500 Gy, potato protoplasts never yielded microcalli. Fusion products were able to grow only on culture media that contained both hygromycin and kanamycin. Altogether 47 "150-Gy" and 63 "500-Gy" hybrid fusion calli were recovered from fusion experiments Xa11-H8 (+) 7322-K5, 150-Gy irradiated, and Xa11-H4 (+) 7322-K5, 500-Gy irradiated, respectively. Regeneration of somatic hybrids was inhibited by gamma irradiation of the potato protoplasts prior to fusion: 38% of the 150-Gy calli and 23% of the 500-Gy calli formed small shootlike structures within 18 months after fusion. Symmetric "0-Gy" fusion products, selected in the same way, showed an earlier regeneration at a significantly higher frequency (approximately 95% within 18 months after fusion). Most asymmetric somatic hybrid shoots showed gross morphological abnormalities and grew poorly as compared to symmetric hybrids. None of the shoots from asymmetric fusion experiments could be rooted in vitro on MS20 medium and transferred to the greenhouse. Nearly all shoots from symmetric fusion experiments could be rooted on MS20 medium and grown in the greenhouse.

Fluorometric GUS assay

All calli, selected on media that contained both hygromycin and kanamycin, displayed GUS activity (a trait derived from potato and absolutely linked to kanamycin resistance), which confirmed the hybridity of the calli. Half a year after fusion, the selected calli were removed from the selection medium and were subsequently subcultured every 2 months on medium without antibiotics for half a year. During this time all calli retained their GUS activity (GUS activity was determined every 3 months), which suggests that limited further elimination of potato DNA took place under these non-selective conditions.

Flow cytometric and nuclear DNA analysis

Flow cytometric analysis of approximately 1 year-old hybrid calli showed that the genomic C-value, which corresponds to the ploidy level of both tomato and potato, was highly variable between calli. Nearly all calli were hyper-tetraploid. For the 150-Gy calli the average ploidy level was 10.0 (ranging from 6.0 to 18.6); for the 500-Gy calli this was 7.6 (ranging from 3.9 to 18.2). Some preference could be observed for ploidy levels that were multiples of four (Figs. 1, 4). The genomic DNA constitution, calculated from dot blots, was also highly variable between fusion calli. For the 150-Gy calli the average estimated percentage of potato nuclear DNA was 12.6 (ranging from 0.9 to 25.8). For the 500-Gy calli this was 10.3 (ranging from 0.3 to 39.9).

Estimated numbers of tomato and potato genome-equivalents of individual calli are presented in Fig. 1. The fact that both the average ploidy level and the average percentage of potato nuclear DNA of the 500-Gy calli were lower than that of the 150-Gy calli demonstrates a stronger elimination of potato nuclear DNA following 500-Gy irradiation.

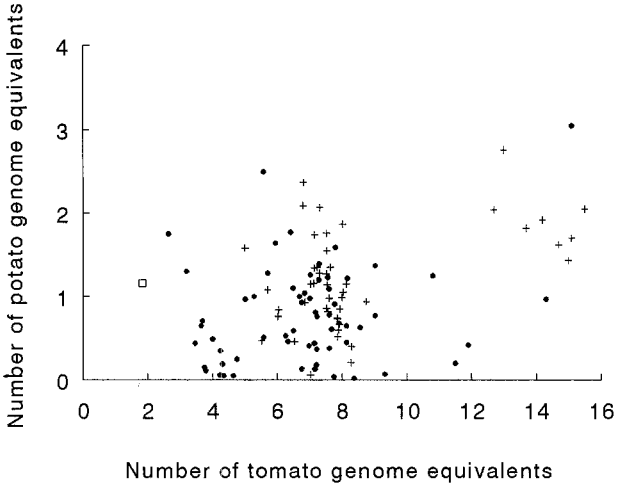

Fig. 1. Estimated numbers of potato and tomato genome equivalents of individual 150-Gy (+) and 500-Gy (o) calli, calculated from C-values and dot-blot hybridization signals. A C-value of 1 corresponds with one complement of 12 tomato or potato chromosomes. An allotriploid somatic hybrid (A7-146D, described by Schoenmakers et al. (1993), of a diploid tomato (with a C-value of 2) and a monohaploid potato (with a C-value of 1) was used as a reference source (□)

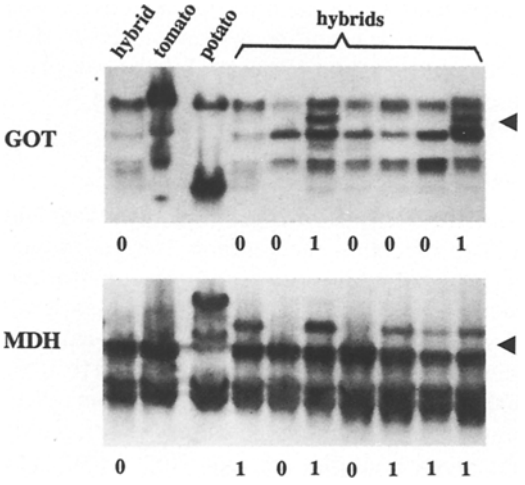

Fig. 2. GOT and MDH zymograms of tomato, potato, and some asymmetric fusion products. The heterodimeric band (◀) is present (= 1) or absent (= 0)

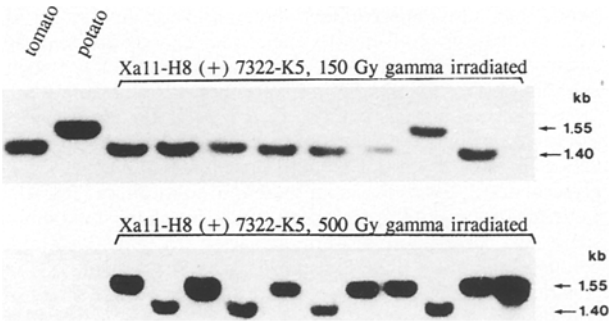

Fig. 3. Identification of chloroplast types in fusion products. RFLP analysis of total DNA of parental and hybrid tissue with the chloroplast clone pPCY64 of *P. hybrida* used as a probe. All lanes contain 4 µg of *Hin*fI-digested total DNA

Isoenzyme analysis

The absence of heterodimeric, hybrid GOT, and/or MDH, bands is an indication of the elimination of potato nuclear DNA (Fig. 2). Of the 150-Gy calli, 7% did not express a potato GOT isoenzyme while 31% did not express a potato MDH isoenzyme. For the 500-Gy calli these figures were 53% and 56% respectively. Tomato-specific bands were always present.

Chloroplast DNA analysis

Restriction fragment length polymorphism (RFLP) analysis of total DNA of hybrid calli with the *P. hybrida* chloroplast clone pPCY64 suggests that calli contain either tomato or potato chloroplasts (Fig. 3). No novel restriction patterns were found. Of the 150-Gy calli, 96% possessed tomato chloroplasts. For the 500-Gy calli this preference was not found: 51% of the hybrids contained tomato chloroplasts.

Relations between various genetic parameters

Relations between shoot regeneration, the flow cytometric C-value, the nuclear DNA constitution, the presence or absence of the heterodimeric isoezymes for GOT and MDH, and the chloroplast type, are presented in Fig. 4 and Table 1 for both doses. Remarkably, many of the genetic parameters behave independently. Only for the 150-Gy calli was the retainment of the potato MDH isoenzyme significantly stronger at higher ploidy levels. Furthermore, 500-Gy calli with a lower percentage of potato nuclear DNA displayed a significantly-better shoot regeneration. The presence or absence of potato GOT and potato MDH were correlated for the 500-Gy calli.

Table 1. Chi-square values for a test of independence between the various genetic parameters: the regeneration (REG), the ploidy level (PLOI) [higher or lower than 12 for 150-Gy calli and higher or lower than average (= 7.6) for 500-Gy calli], the nuclear DNA constitution (NDC) (higher or lower than the average percentage of potato DNA), the presence or absence of the heterodimeric isoenzymes for GOT and MDH, and the chloroplast type (CP) (tomato or potato) for the fusion products Xa11-H8 (+) 7322-K5, 150-Gy irradiated and Xa11-H4 (+) 7322-K5, 500-Gy irradiated [$P(\chi_1^2 > 3.84) < 0.05$; $P(\chi_1^2 > 6.64) < 0.01$]. Significant correlations are indicated with *($P < 0.05$) or **($P < 0.01$)

Parameter	150-Gy calli					500-Gy calli				
	REG	PLOI	NDC	GOT	MDH	REG	PLOI	NDC	GOT	MDH
PLOI	3.03					0.89				
NDC	3.74	1.53				10.14**	1.42			
GOT	1.77	0.36	2.35			0.11	0.00	1.54		
MDH	1.85	5.08*	0.00	1.90		1.65	1.27	1.48	5.03*	
CP	3.69	0.49	3.08	0.15	0.35	3.07	0.77	0.77	0.06	0.59

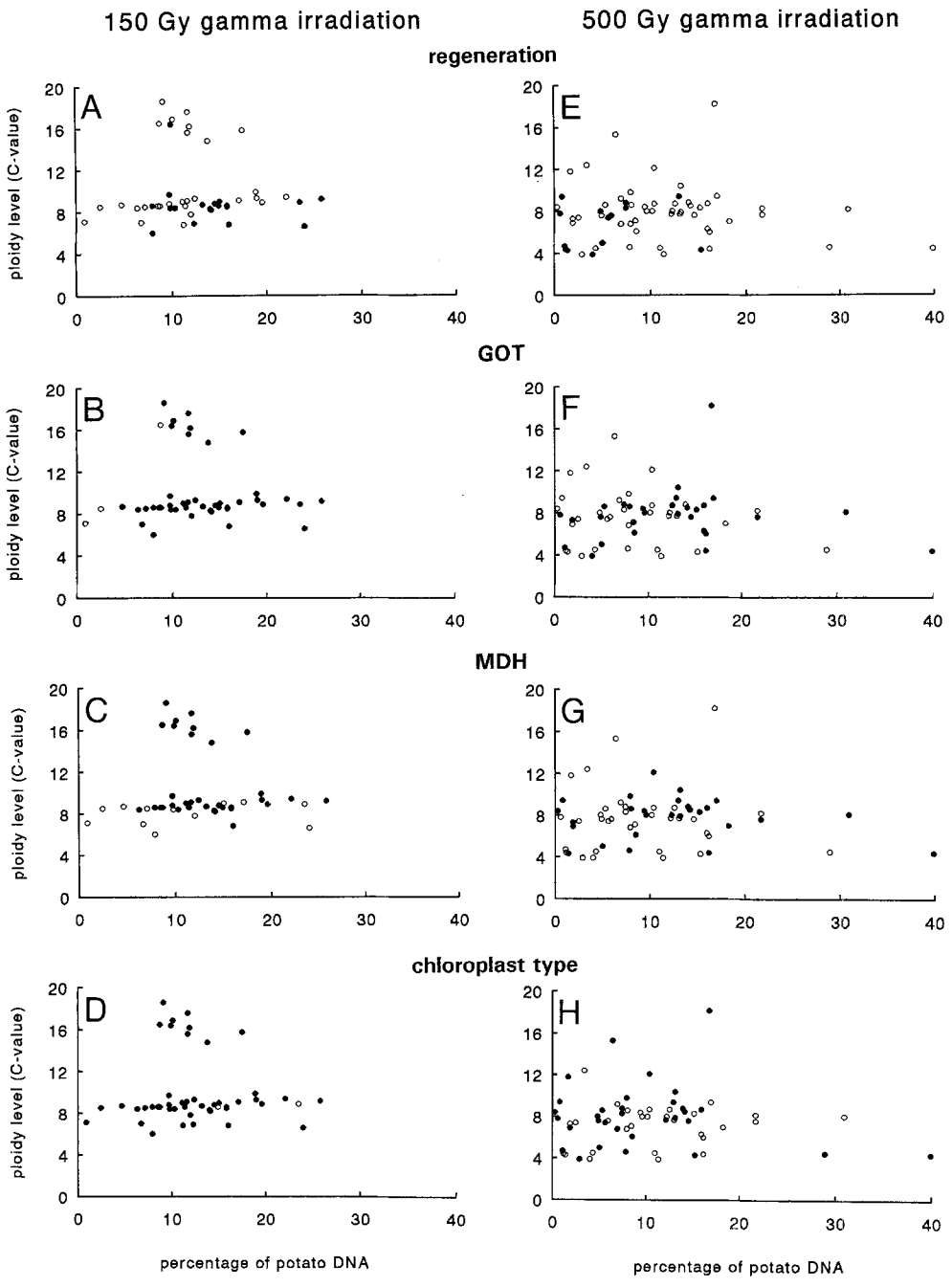

Fig. 4. Relations between the percentage of potato nuclear DNA in individual 150-Gy hybrid calli, the ploidy level and **a** the regeneration (● = regeneration; ○ = no regeneration), **b** the presence (●) or absence (○) of potato GOT, **c** the presence (●) or absence (○) of potato MDH and **d** the presence of tomato (●) or potato (○) chloroplasts. For 500-Gy calli these relations are shown in **e**, **f**, **g** and **h** respectively

Discussion

Asymmetric somatic hybrids between tomato and potato were selected only on the basis of their ability to grow at least as callus. The analysis of seven genetic parameters of these hybrids shows that fertile asymmetric hybrid plants, with a relatively strong elimination of donor DNA, are very difficult to obtain under the experimental conditions that we used. The irradiation of the donor prior to protoplast fusion caused elimination of donor traits and donor nuclear DNA. We found that with 500-Gy gamma irradiation the

elimination of potato isoenzymes for GOT and MDH, and the elimination of potato nuclear DNA, was stronger than with 150-Gy irradiation. This radiation dose-dependent elimination of donor traits has also been described by Bonnema et al. (1992), Melzer and O'Connell (1992) and Menczel et al. (1992) for other solanaceous species.

In the 150-Gy calli the potato MDH was more frequently eliminated than the potato GOT. Possibly, the potato GOT gene is more closely linked to a potato trait which plays an important role in the development of the fusion product or to the kanamycin-resistance gene. Chances for elimination might also depend on the location on the chromosome. Although centromeres are necessary for retainment of the donor chromosomes and/or chromosome fragments, extensive rearrangements, due to the irradiation treatment, may result in more distal chromosome regions with nearby centromere fragments. Furthermore, damaged DNA fragments can be 'rescued' by somatic recombination with recipient chromosomes (Piastuch and Bates 1990; Parokonny et al. 1992). The fact that in the 500-Gy calli the elimination of the potato GOT was as strong as that of the potato MDH, whereas in the 150-Gy calli the potato GOT was more frequently retained than the potato MDH, suggests that, apart from an increased donor DNA elimination following 500-Gy irradiation, more donor chromosome breakage and/or rearrangements have taken place after 500-Gy gamma irradiation. Indications for extensive donor chromosome rearrangements after irradiation were already reported by Wijbrandi et al. (1990a) and Parokonny et al. (1992).

In all hybrid calli the recipient DNA amount has increased considerably, irrespective of the percentage of potato DNA. This polyploidization of the recipient genome after asymmetric fusion was also observed by Gleba et al. (1988) and Wijbrandi et al. (1990a). Possibly, polyploidization of the recipient tomato genome is necessary to buffer the negative effect of the additional genetic material of the donor and the aneuploidy.

The observation that the percentage of potato DNA was correlated neither with the regeneration nor with the ploidy level suggests that it should be possible to select highly-asymmetric somatic hybrids of tomato and potato when large numbers of somatic hybrids are available. However, the polyploidization of the tomato genome, which was observed in all hybrid calli, might be inevitable and poses an important restriction to the use of asymmetric somatic hybridization for plant breeding in this species combination. Sexual hybridization between solanaceous genotypes differing in ploidy level is extremely difficult.

Recent studies of asymmetric somatic hybridization between *Lycopersicon esculentum* as a recipient and *Solanum* species as a donor suggest that a certain amount of *Solanum* DNA is required to establish functional *Solanum* chloroplasts in the fusion product (Wolters et al. 1991; Derks et al. 1992). However, we did not observe a stronger retention of potato DNA in calli with potato chloroplasts in our experiments. We can only conclude that the equivalent of approximately one potato chromosome is sufficient to establish potato chloroplasts. Because rearrangements occur frequently in these asymmetric hybrids (see above) it is possible that this chromosome equivalent represents parts from different chromosomes.

From earlier studies we know that chloroplasts in symmetric and asymmetric fusion products of *Lycopersicon* and *Solanum* species sort out to homogeneity for either parent during cell division (Schiller et al. 1982; O'Connell and Hanson 1985; Levi et al. 1988). The results obtained for the 500-Gy calli indicate that sorting out of tomato and potato chloroplasts was at random. This observation matches the data of Derks et al. (1991), who showed that irradiation did not lead to a significant reduction of the irradiated chloroplast genome in somatic hybrids of *L. esculentum* and *L. peruvianum* which were irradiated with 50-, 300- or 1000-Gy, or left unirradiated. However, it contradicts the observation of Bonnema et al. (1992) that sorting out was radiation-dose dependent in somatic hybrids of *L. esculentum* and *L. pennellii* which were irradiated with 50-, 100-, 150-, 250-, 500- or 1000-Gy, or left unirradiated. Bonnema et al. (1992) also reported that the chloroplast genotype of the asymmetric fusion products reflected the predominant nuclear genotype. We did not observe this correlation in either hybrid series. No explanation can be given for the almost complete absence of potato chloroplast DNA in the 150-Gy hybrids.

Apart from a possible irradiation-dependent and nuclear composition-dependent sorting out, the symmetric fusion experiments of Pehu et al. (1989) and Li and Sink (1992) and the asymmetric fusion experiments of Bonnema et al. (1992) demonstrated that the direction in which sorting out of chloroplasts takes place is strongly dependent on undefined differences in experimental conditions. Because our 150-Gy and 500-Gy fusions were carried out at different times with different transformants of one tomato genotype, the two callus populations cannot be compared with respect to their chloroplast constitution.

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References

Arumuganathan K, Earle ED (1991) Nuclear DNA content of some important plant species. Plant Mol Biol Rep 9:208–218

Bates GW (1990) Asymmetric hybridization between *Nicotiana tabacum* and *N. repanda* by donor recipient protoplast fusion: transfer of TMV resistance. Theor Appl Genet 80:481–487

Bonnema AB, Melzer JM, Murray LW, O'Connell MA (1992) Non-random inheritance of organellar genomes in symmetric and asymmetric somatic hybrids between *Lycopersicon esculentum* and *L. pennellii*. Theor Appl Genet 84:435–442

Derks FHM, Wijbrandi J, Koornneef M, Colijn-Hooymans CM (1991) Organelle analysis of symmetric and asymmetric hybrids between *Lycopersicon peruvianum* and *Lycopersicon esculentum*. Theor Appl Genet 81:199–204

Derks FHM, Hakkert JC, Verbeek WHJ, Colijn-Hooymans CM (1992) Genome composition of asymmetric hybrids in relation to the phylogenetic distance between the parents. Nucleus-chloroplast interaction. Theor Appl Genet 84:930–940

Dudits D, Fejér O, Hadlaczky G, Koncz C, Lázár GB, Horváth G (1980) Intergeneric gene transfer mediated by plant protoplast fusion. Mol Gen Genet 179:283–288

Dudits D, Maroy E, Praznovsky 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 *Nicotiana plumbaginifolia* and *Atropa belladonna* obtained by "gamma-fusion". Theor Appl Genet 76:760–766

Hinnisdaels S, Bariller L, Mouras A, Sidorov V, Del-Favero J, Veuskens J, Negrutiu I, Jacobs M (1991) Highly-asymmetric intergeneric nuclear hybrids between *Nicotiana* and *Petunia*: evidence for recombinogenic and translocation events in somatic hybrid plants after "gamma"-fusion. Theor Appl Genet 82:609–614

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

Itoh K, Futsuhara Y (1983) Interspecific transfer of only part of genome by fusion between non-irradiated protoplasts of *Nicotiana glauca* and X-ray-irradiated protoplasts of *N. langsdorffii*. Jpn J Genet 58:545–553

Jefferson RA, Kavanagh TA, Bevan MW (1987) GUS fusions: β-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. EMBO J 6:3901–3907

Koornneef M, Hanhart CJ, Martinelli L (1987a) A genetic analysis of cell-culture traits in tomato. Theor Appl Genet 74:633–641

Koornneef M, Jongsma M, Weide R, Zabel P, Hille J (1987b) Transformation of tomato. In: Nevins DJ, Jones RA (eds) Tomato biotechnology. Alan R Liss Inc, New York, pp 169–178

Koornneef M, Bade J, Hanhart C, Horsman K, Schel J, Soppe W, Verkerk R, Zabel P (1993) Characterization and mapping of a gene controlling shoot regeneration in tomato. The Plant J 3:131–141

Levi A, Ridley BL, Sink KC (1988) Biased organelle transmission in somatic hybrids of *Lycopersicon esculentum* and *Solanum lycopersicoides*. Curr Genet 14:177–182

Li Y, Sink KC (1992) Cell type determines plastid transmission in tomato intergeneric somatic hybrids. Curr Genet 22:167–171

Melzer JM, O'Connell MA (1992) Effect of radiation dose on the production of and the extent of asymmetry in tomato asymmetric somatic hybrids. Theor Appl Genet 83:337–344

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

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

O'Connell MA, Hanson MR (1985) Somatic hybridization between *Lycopersicon esculentum* and *Solanum pennellii*. Theor Appl Genet 70:1–12

Parokonny AS, Kenton AY, Gleba YY, Bennett MD (1992) Genome reorganization in *Nicotiana* asymmetric somatic hybrids analysed by in-situ hybridization. The Plant J 2:863–874

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

Persson AR (1960) Physiological and genetical investigations on chlorophyll mutants. Rep Tomato Genet Coop 10:27–28

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

Sacristan MD, Gerdemann-Knörck, Schieder O (1989) Incorporation of hygromycin resistance in *Brassica nigra* and its transfer to *B. napus* through asymmetric protoplast fusion. Theor Appl Genet 78:194–200

Schiller B, Herrmann RG, Melchers G (1982) Restriction endonuclease analysis of plastid DNA from tomato, potato and some of their somatic hybrids. Mol Gen Genet 186:453–459

Schoenmakers HCH, Nobel EM, Koornneef M (1992) Somatic hybridization between nitrate-reductase-deficient mutants of tomato (*Lycopersicon esculentum* Mill.) and wild-type potato (*Solanum tuberosum* L.) Plant Cell Tissue Org Cult 31: 151–154

Schoenmakers HCH, Wolters AMA, Nobel EM, Klein CMJ de, Koornneef M (1993) Allotriploid somatic hybrids of diploid tomato (*Lycopersicon esculentum* Mill.) and monoploid potato (*Solanum tuberosum* L.) Theor Appl Genet (in press)

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

Somers DA, Naterayan 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

Vallejos CE (1983) Enzyme activity staining. In: Tanksley SD, Orton TJ (eds), Isozymes in plant genetics and breeding, part A. Elsevier, Amsterdam, pp 469–515

Visser RGF, Hoekstra R, Van der Leij FR, Pijnacker LP, Witholt B, Feenstra WJ (1988) In-situ hybridization to somatic metaphase chromosomes of potato. Theor Appl Genet 76:420–424

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

Wolters AMA, Schoenmakers HCH, van der Meulen-Muisers JJM, van der Knaap E, Derks FHM, Koornneef M, Zelcer A (1991) Limited DNA elimination from the irradiated potato parent in fusion products of albino *Lycopersicon esculentum* and *Solanum tuberosum*. Theor Appl Genet 83:225–232

Wolters AMA, Koornneef M, Gilissen LJW (1993) The chloroplast and the mitochondrial DNA type are correlated with the nuclear composition of somatic hybrid calli of *Solanum tuberosum* and *Nicotiana plumbaginifolia*. Curr Genet 24: 260–267

Wijbrandi J, Zabel P, Koornneef M (1990a) 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

Wijbrandi J, Wolters AMA, Koornneef M (1990b) Asymmetric somatic hybrids between *Lycopersicon esculentum* and irradiated *Lycopersicon peruvianum*. Theor Appl Genet 80: 665–672

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

Zabel P, Meyer D, Van de Stolpe O, Van der Zaal B, Ramanna MS, Koornneef M, Krens F, Hille J (1985) Towards the construction of artificial chromosomes for tomato. In: van Vloten-Doting L, Groot GSP, Hall TC (eds), Molecular form and function of the plant genome. Plenum, New York, pp 609–624