Theor Appl Genet (1990) 80:665–672

Asymmetric somatic hybrids between *Lycopersicon esculentum* and irradiated *Lycopersicon peruvianum*

2. Analysis with marker genes

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Received April 19, 1990; Accepted June 1, 1990
Communicated by P. Maliga

Summary. Asymmetric somatic hybrids of *Lycopersicon esculentum* and *Lycopersicon peruvianum* were analysed for the retention of genes and alleles specific for *L. peruvianum*. The hybrids were obtained by fusion of protoplasts from *L. esculentum* with those of *L. peruvianum* (the donor), the latter having been irradiated before fusion with 50, 300 or 1,000 Gy of gamma-rays. The retention of three different types of genes or alleles was analysed. (1) The gene coding for kanamycin resistance, which is dominant and had been introduced in most of the *L. peruvianum* donor plants by transformation. It was present at one locus in 16 *L. peruvianum* donor plants and at two loci in one donor plant. (2) The genes coding for acid phosphatase, locus *Aps-1,* and glutamate oxaloacetate transaminase (GOT); different alleles of these genes are co-dominant and were detected by isozyme analysis. (3) Eighteen single gene morphological markers for which most of the *L. esculentum* genotypes used were homozygous recessive. Kanamycin resistance from donor plants with one locus was retained in about 50% of the asymmetric 30H-hybrids (the donor was irradiated with 300 Gy). *L. peruvianum* specific alleles of *Aps-1* and GOT were present in at least 70% of the hybrids; the retention of donor alleles was lower in 30H- than in 5H-hybrids (donor irradiated with 50 Gy). On average, 73% of the *L. peruvianum*-specific alleles (one or both) of the morphological markers were detected in the 30H-hybrids. Several of the *L. esculentum* genotypes used were homozygous recessive for two morphological markers on the same chromosome; in 43% of the 30H-hybrids derived from them, only one of these markers was complemented by the *L. peruvianum* allele. This is an indication of frequent breakage of the *L. peruvianum* chromosomes. Several hybrid calli regenerated genotypically different shoots. On the whole, this analyses confirms the conclusion drawn from the cytogenetic and morphological analysis of these asymmetric hybrids, namely that irradiation prior to fusion eliminates the *L. peruvianum* genome to only a limited extent.

Key words: Tomato – Protoplast fusion – Gamma irradiation – Isozyme analysis – Morphological markers – Partial genome transfer

Introduction

Partial genome transfer by asymmetric somatic hybridisation, which involves the fusion of protoplasts of a recipient species with irradiated protoplasts of a donor species, has been described for several plants. The transferred donor genome has varied from a few traits (Dudits et al. 1987) to many chromosomes (Gleba et al. 1988; Famelaer et al. 1989; Yamashita et al. 1989). The amount of transferred donor genome was often assessed on the basis of chromosome counts. This is not always an accurate estimation, because discrimination between the species-specific chromosomes and identification of incomplete chromosomes are not always possible.

In a previous paper (Wijbrandi et al. 1990c) we described the cytogenetic and morphological analysis of a series of asymmetric hybrids of *L. esculentum* (the cultivated tomato) and *L. peruvianum* (a wild species; the donor). These hybrids appeared to still contain a relatively large number of *L. peruvianum* chromosomes. In most of the asymmetric hybrids the chromosome number was around the triploid or pentaploid level; the genome of

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these hybrids probably consisted of a diploid or tetraploid genome from *L. esculentum* and several chromosomes from *L. peruvianum*. The amount of donor chromosomes varied and did not correlate strongly with the irradiation dose applied to the *L. peruvianum* protoplasts (50, 300 and 1,000 Gray, respectively). To study the elimination of the genetic material of the donor species in more detail, the same asymmetric hybrids were analysed for transfer of single gene markers. This kind of analysis can be performed very well in the tomato because of its detailed linkage map. The map consists of more than 300 morphological, isozyme- and disease-resistance genes (Mutschler et al. 1987) and at least 300 RFLP markers (Young and Tanksley 1989). We tried to estimate the fraction of the *L. peruvianum* genome that was conserved in the asymmetric somatic hybrids by analysing the retention of three types of genes or alleles:

(1) the neomycin phosphotransferase II (NPT II) gene, which causes resistance to the antibiotic kanamycin. This is a dominant gene that had been introduced into the nuclear genome of most of the *L. peruvianum* donor plants by transformation;
(2) the isozyme markers acid phosphatase (*Aps-1*) and glutamate oxaloacetate transaminase (GOT);
(3) single gene-determined morphological markers present in most of the tomato genotypes used.

Material and methods

The genotypes of *L. esculentum*, the recipient species, were the Dutch hybrid cv 'Bellina' (kindly provided by Rijk Zwaan Seed Company, de Lier, The Netherlands) and seven multiple marker lines (Table 1) from the Tomato Genetics Stock Center in Davis, Calif., USA (kindly provided by Prof. C. M. Rick). Plants from the *L. peruvianum* accession PI128650 (received from the Institute of Horticultural Plant Breeding, Wageningen, The Netherlands) were used as the donor. Seventeen kanamycin-resistant *L. peruvianum* plants, obtained by leaf disc transformation with *A. tumefaciens* containing the plasmid pAGS112 (Koornneef et al. 1987b), were available; these plants were designated ATW2001–ATW2027. The isolation of the asymmetric somatic hybrids has been described previously (Wijbrandi et al. 1990c). The asymmetric hybrids were designated according to the irradiation dose applied to *L. peruvianum* before protoplast fusion: 5H-, 30H- and 100H-hybrids, which were irradiated with a dose of 50, 300 and 1,000 Gy of gamma-rays, respectively.

Kanamycin resistance assays

To determine the number of NPT II loci in the independent *L. peruvianum* transformants, the transformants were backcrossed as pistillate parent to the wild-type *L. peruvianum*. The resulting seeds were decontaminated by treatment in 70% ethanol (10 sec) and 5 × diluted commercial bleach (10% NaClO) (20 min), and washed several times in sterile water. The seeds were transferred to plastic containers with shoot culture medium (Wijbrandi et al. 1990a) supplemented with 100 mg/l kanamycin and incubated in the dark. After a few days the seeds germinated and were transferred to light conditions. Two weeks later the seedlings were scored for growth (= resistance).

Table 1. The multiple marker lines of *L. esculentum* used in the present study. The morphological marker genes are given with their chromosomal location (Rick 1982). * Indicates a character that was used in the analysis of the asymmetric somatic hybrids of *L. esculentum* (recipient) and *L. peruvianum* (donor)

Tomato genotype	Markers [chromosome arm]
LA291	*ms-2* (=male sterile) [2L]
	* *hl* (=hairless, no large trichomes) [11S]
	* *a* (=anthocyaninless) [11L]
LA1164	* *var* (=variabilis, leaves emerge yellow) [7S]
	* *not* (=notabilis, leaves wilting) [7L]
	* *ah* (=anthocyaninless) [9L]
	* *marm* (=marmorata, leaves marbled white-green) [9L]
LA1166	* *clau* (=clausa, leaves subdivided) [4S]
	* *di* (=divergens, stems slender and whitish) [4L]
	icn (=incana, leaves with whitish margins) [10S]
	ag (=anthocyanin gainer, laminae abaxially pigmented) [10L]
LA1182	* *sy* (=sunny, leaves emerge yellow) [3S]
	* *sf* (=solanifolia, leaflets entire and concave) [3L]
	* *alb* (=albescent, strong white-green variegation) [12S]
	mua (=multifurcata, dull green interveinal chlorosis) [12L]
LA1189	* *yv* (=yellow virescent, leaves emerge yellow) [6L]
	* *c* (=potato leaf, fewer leaf segments) [6L]
LA1444	* *af* (=anthocyaninless) [5S]
	tf (=trifoliate, leaves 3-segmented) [5S]
	wv (=white virescent, leaves emerge white) [2L]
	d (=dwarf) [2L]
LA1665	* *dgt* (=diageotropica, stems and roots diageotrophic) [1L]
	* *l* (=lutescent, leaves yellowing) [8S]
	* *al* (anthocyanin loser, pigmented only at nodes later) [8L]

To determine kanamycin resistance in calli, which were derived from protoplast cultures of a *L. esculentum* genotype and one of the *L. peruvianum* ATW-plants (with and without fusion treatment), we sliced the calli and subcultured one part on the callus medium TMc (Wijbrandi et al. 1990a) and transferred the other part to TMc supplemented with 100 mg/l kanamycin. Kanamycin-sensitive calli turned brown on the latter medium. To test kanamycin resistance of somatic hybrid plants, cuttings of hybrid shoots were transferred to shoot culture medium supplemented with 100 mg/l kanamycin: sensitive shoots formed no roots and appeared bleached after a few weeks.

Southern blot analysis

DNA was isolated from several plants according to Dellaporta et al. (1983), digested with *Dra*I, separated by agarose gel electrophoresis, blotted onto Gene Screen PLUS (New England Nuclear) and hybridised with a probe for the NPT II gene. As probe was used the *Cla*I-*Sal*I fragment, which contained the NOS-promoter and the structural gene of the T-region of plasmid pAGS112 (kindly provided by Dr. P.J.M. van den Elzen, MOGEN, Leiden, The Netherlands). The hybridisation procedure has been described earlier (Wijbrandi et al. 1990b).

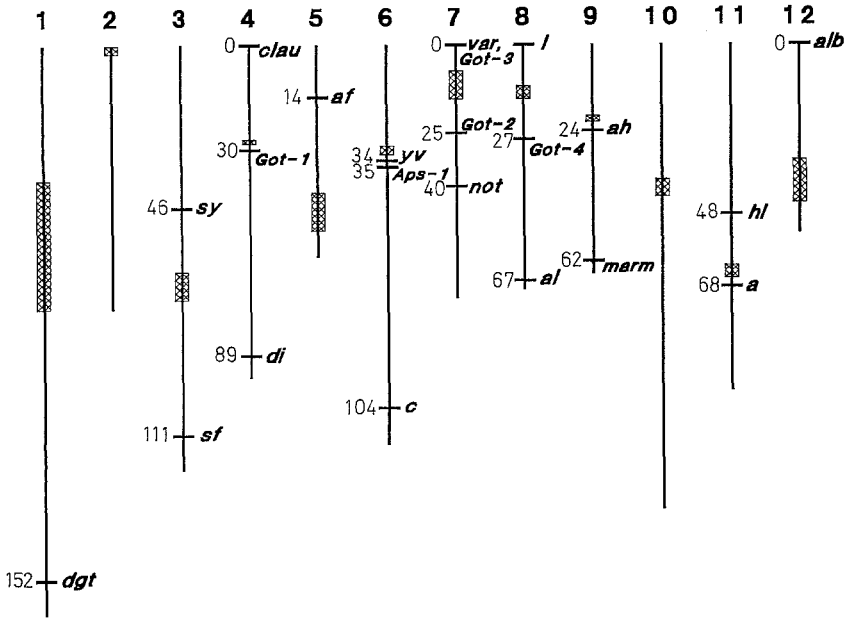

Fig. 1. Linkage map of the morphological and isozyme markers used in the analysis of the asymmetric somatic hybrids. The *shaded areas* indicate the centromeres. All positions are according to Mutschler et al. (1987)

Isozyme analysis

Leaf material from greenhouse-grown plants was used for the analysis of acid phosphatase, locus *Aps-1,* and glutamate oxaloacetate transaminase (GOT), loci *Got-1, Got-2, Got-3* and *Got-4;* the positions of these loci on the linkage map of tomato are shown in Fig. 1. Crude extracts and extracts prepared according to Suurs et al. (1989), were electrophoresed on vertical polyacrylamide slab gels (Wijbrandi et al. 1990a). Enzyme activity was stained according to Vallejos et al. (1983).

Morphological markers

Figure 1 shows the position of the recessive morphological markers that were used in the present study on the linkage map of *L. esculentum.* The *L. esculentum* genotypes that were used for the fusions were homozygous for two–four of these markers located on one–two different chromosomes (Table 1). The asymmetric hybrids, preferably greenhouse-grown plants, were assayed for the phenotype of the relevant markers to determine the retention of the dominant *L. peruvianum* wild-type alleles. The presence of some markers could not be assayed because they were typical hypocotyledon markers (e.g. *ag*) or because the hybrid and aneuploid nature of the hybrids interfered with the expression of the mutant phenotype.

Results

Number of kanamycin resistance loci in L. peruvianum plants

The segregation ratios of kanamycin resistance in the testcross of *L. peruvianum* ATW-plants with wild-type *L. peruvianum* are given in Table 2. Sixteen ATW-plants showed a ratio of 1:1, which indicates the presence of one NPT II locus, whereas one plant, ATW2002, had a ratio of 3:1, which indicates that NPT II genes were inserted at two different unlinked loci. This was confirmed by Southern blot analysis using the NPT II gene as probe (Fig. 2): ATW2002 showed two distinct fragments, while

Table 2. Segregation of kanamycin resistance in the testcross of kanamycin-resistant *L. peruvianum* PI128650 plants (ATW2001–ATW2011, ATW2014–ATW2016, ATW2020, ATW2021 and ATW2027) as female parent with wild-type *L. peruvianum* PI128650, and among regenerating 30H-hybrid calli, which derived from protoplast fusions between *L. esculentum* and 300 Gy gamma-irradiated kanamycin-resistant *L. peruvianum.* The assays were performed on media supplemented with 100 mg/l kanamycin. The calli were assayed before regeneration occurred

L. peruvianum genotype	Segregation ratio in testcross		Ratio in 30H-calli $Km^R:Km^S$
	$Km^R:Km^S$	$[\chi^2\{1:1\}]$[a]	
ATW2001	29:35	[0.56]	1:3
ATW2003	35:29	[0.56]	1:0
ATW2004	32:32	[0.00]	9:3
ATW2005	73:53	[3.17]	6:2
ATW2006	41:53	[1.53]	2:1
ATW2007	31:33	[0.06]	1:0
ATW2008	36:27	[1.29]	3:0
ATW2009	33:32	[0.02]	7:5
ATW2010	33:31	[0.06]	3:0
ATW2011	31:33	[0.06]	4:1
ATW2014	59:66	[0.39]	5:3
ATW2015	31:33	[0.06]	1:3
ATW2016	29:34	[0.40]	1:1
ATW2020	29:33	[0.26]	13:1
ATW2021	31:41	[1.39]	5:3
ATW2027	32:32	[0.00]	3:2
		total	65:28
ATW2002	47:17	[14.06][b]	

Km^R, Kanamycin-resistant; Km^S, kanamycin-sensitive

[a] Segregation ratio does not deviate significantly from 1:1, if $\chi^2 < \chi^2(1, 0.95) = 3.84$; consistent with the presence of one NPT II locus

[b] Segregation ratio does not deviate significantly from 3:1 ($\chi^2 = 0.08$); consistent with two unlinked NPT II loci

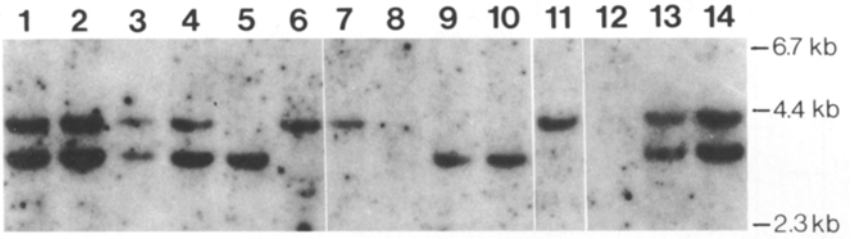

Fig. 2. Southern blot analysis of some somatic hybrids and their parents with a probe of the NPT II gene. *Dra*I digests of the following genotypes were analysed: *lane 1* 5H2, *lane 2* 5H3, *lane 3* 5H5, *lane 4* 5H7, *lane 5* 5H13, *lane 6* 5H16, *lane 7* 5H28, *lane 8* 5H10, *lane 9* 30H1, *lane 10* 30H3, *lane 11* 30H7, *lane 12 L. esculentum* cv 'Bellina', *lane 13* 0H1, *lane 14 L. peruvianum* PI128650-ATW2002. All hybrids were derived from protoplast fusions between *L. esculentum* cv 'Bellina' and ATW2002. The latter was either unirradiated (symmetric hybrid 0H1), irradiated with 50 Gy of gamma-rays (asymmetric hybrids 5H2–5H28) or irradiated with 300 Gy of gamma-rays (asymmetric hybrids 30H1–30H7) before fusion. The position of the *Hin*dIII fragments of phage lambda DNA is indicated at the *right*

the tested kanamycin-resistant hybrids of 'Bellina' (+) ATW2002, namely one symmetric and ten asymmetric hybrids, had either one or both of these bands, which segregated independently. The only tested kanamycin-sensitive hybrid of the same parental genotypes, 5H10, had no such band(s).

Retention of kanamycin resistance loci in asymmetric hybrids

The ratio of kanamycin-resistant to kanamycin-sensitive regenerating calli derived from fusions of *L. esculentum* and irradiated *L. peruvianum* containing one NPT II locus is shown in Table 2 (right column): 70% of the tested calli (65 out of 93) were kanamycin resistant. The assay was repeated for shoots that had regenerated from 12 resistant 30H-calli. Only a minority of the 30H-hybrids were tested at the plant level because of the very low growth rate of many of these hybrids and because we preferred to transfer the shoots to the greenhouse. Shoots from 7 of these 12 resistant calli were still resistant, whereas the shoots from 4 resistant calli were kanamycin sensitive. From another resistant hybrid callus one resistant and one sensitive shoot was regenerated. Thus, in one-third of the 30H-hybrids that were kanamycin resistant at the callus level, the resistance was lost in the shoots. Assuming that the chance to lose this trait was the same in all resistant 30H-hybrids, we estimate that 46% ($\approx$43 out of 93) of the 30H-hybrids had retained the kanamycin resistance from *L. peruvianum*.

The retention of kanamycin resistance in nine 30H-hybrids (all nine tested at the plant level) derived from ATW2002 (with two NPT II loci) was higher: all shoots from eight hybrids were resistant, and those from one hybrid were sensitive.

Isozyme analysis

Both parental species, *L. esculentum* and *L. peruvianum*, had different isozyme patterns (Fig. 3) for *Aps-1*, a locus

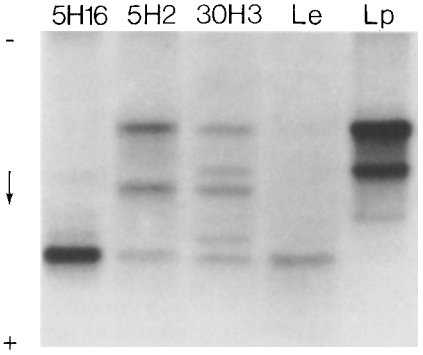

Fig. 3. Isozyme patterns of acid phosphatase, locus *Aps-1*, from left to right of asymmetric somatic hybrids 5H16, 5H2 and 30H3, *L. esculentum* cv 'Bellina' (*Le*) and *L. peruvianum* PI128650 (*Lp*). The asymmetric hybrids were derived from protoplast fusions between *L. esculentum* and *L. peruvianum*, irradiated with 50 Gy (5H2 and 5H16) or 300 Gy (30H3) of gamma-rays

which encodes acid phosphatase-1, a dimeric enzyme (Rick 1983). Three different patterns were found in the asymmetric somatic hybrids (Fig. 3): one pattern resembled the tomato pattern with one fast-migrating band; another pattern had six bands, all bands from both species and two novel, presumed heterodimeric, ones; the third pattern consisted of the single band from tomato, one of the *L. peruvianum*-specific bands, and one presumed interspecific heterodimeric band. The latter pattern indicates the presence of a single allele of the *L. peruvianum Aps-1* in the hybrid. The number of asymmetric hybrids which have lost or retained *Aps-1* alleles from the donor species are shown in Table 3. Most of the hybrids contained alleles from the donor species. A larger fraction of the higher dose hybrids (30H and 100H) than of the low dose (5H) hybrids lacked *L. peruvianum* alleles. For two hybrid calli differences between the separate shoots were observed. We could not determine the number of retained donor alleles as the hybrid pattern of six bands can result from either one or two *L. peruvianum* alleles (data not shown).

Table 3. The presence of *L. peruvianum*-specific and hybrid bands in isozyme patterns of asymmetric somatic hybrids of *L. esculentum* and *L. peruvianum* irradiated with 50, 300 and 1,000 Gy of gamma-rays (5H-, 30H- and 100H-hybrids, respectively). The tested isozymes were acid phosphatase, locus *Aps-1*, and glutamate oxaloacetate transaminase (GOT). The GOT bands were assayed in two parts: the two slow-migrating bands and the three fast-migrating bands (see Fig. 4). "Mixed" means that the assayed bands were absent in one shoot from a hybrid callus, whereas these bands were present in another shoot from the same callus. The number of hybrids in which not all *L. peruvianum* and hybrid bands were present is indicated between parentheses

Genotypes	Absent	Present	Mixed
	Aps-1		
5H-hybrids	1	23 (5)	0
30H-hybrids	2	7	1
100H-hybrids	1	0	1 (1)
	"Slow" GOT bands		
5H-hybrids	1	7	0
30H-hybrids	7	14 (8)	3 (2)
100H-hybrids	0	2 (2)	0
	"Fast" GOT bands		
5H-hybrids	1	6 (4)	1 (1)
30H-hybrids	2	20 (8)	2 (1)
100H-hybrids	2	0	0

For glutamate oxaloacetate transaminase (GOT), four loci on three different chromosomes (Fig. 1) are known, and at least three of these are dimeric enzymes (Rick 1983). Isozyme analysis of the cultivated tomato shows four GOT bands, while that of *L. peruvianum* shows five or seven bands, of which two or three occupied the same position as the tomato bands. A typical hybrid pattern, as expressed in "symmetric" somatic hybrids (Wijbrandi et al. 1990a), contained nine bands and is represented in Fig. 4. We could not assess which bands correlated with each of the loci. Therefore, the *L. peruvianum*-specific and hybrid bands were divided into two groups – slow and fast migrating – because these segregated independently. The presence of these bands in the asymmetric hybrids was determined, and as in the case of *Aps-1*, most of the hybrids had retained donor alleles (Table 3). Some hybrid calli had regenerated shoots that differed with respect to the retained donor bands. The loss of slow-migrating GOT bands was dose dependent. The fast bands were retained more often, and the frequency of their loss was not clearly dose dependent. It is probable that products from more than one locus are present in the fast-migrating bands. Four hybrids had a complete hybrid pattern of nine bands, and the patterns of only two hybrids resembled the *L. esculentum* pattern. The latter hybrids do not necessarily lack all *L. peruvianum*-specific alleles since several *L. peruvianum* bands comigrate with *L. esculentum* bands on the polyacrylamide gels.

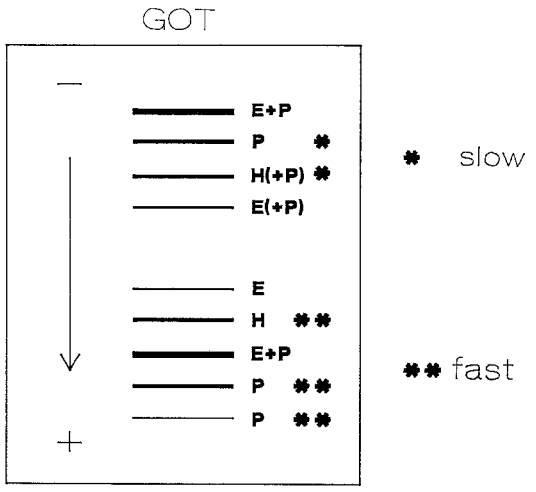

Fig. 4. Representation of the isozyme pattern of glutamate oxaloacetate transaminase found in symmetric somatic hybrids between *L. esculentum* and *L. peruvianum*. At the right side the *L. esculentum*-specific (*E*), the *L. peruvianum*-specific (*P*) and the hybrid-specific (*H*) bands are indicated. The latter two types of bands were divided in slow migrating (*) and fast migrating (**)

Table 4. Complementation of marker genes in asymmetric somatic hybrids of *L. esculentum* and 300 Gy gamma-irradiated *L. peruvianum*. The table shows the number of hybrids having retained *L. peruvianum* allele(s) of each of a series of marker genes. "Absent" means that no complementation was observed in any of the analysed shoots; "Present" means that complementation was observed in all analysed shoots; "Mixed" means that complementation was observed in a number of the analysed shoots of a hybrid. Observations were made on greenhouse-grown plants derived from 71 hybrid calli, and on shoots in vitro. The latter shoots were assayed only for clearly scorable phenotypes

Marker gene	Chromosome position	Corresponding *L. peruvianum* allele(s) in asymmetric hybrids		
		Absent	Present	Mixed
dgt	1–152	1	1	
sy	3– 46	4	4	
sf	3–111	4	6	
clau	4– 0	1	1	
di	4– 89	0	2	1
af	5– 14	0	1	
yv	6– 34	0	2	
c	6–104	3	0	
var	7– 0	0	4	
not	7– 40	0	2	1
l	8– 0	0	1	
al	8– 67	1	5	
ah	9– 24	4	6	
marm	9– 62	0	3	1[a]
hl	11– 48	1	12	2
a	11– 68	6	9	
alb	12– 0	1	5	2[b]
	total	26	64	7

[a] A few sectors of one plant were *marm*
[b] One shoot in vitro of each hybrid was *alb*

Table 5. Complementation of *L. esculentum* marker genes located on a same chromosome by corresponding *L. peruvianum* alleles. Observations were carried out on asymmetric somatic hybrids of *L. esculentum* and *L. peruvianum* irradiated with 300 Gy. Only those plants were included where both markers could be scored unambiguously. The gene symbols supplemented with $^+$ indicate the presence of the wild-type gene derived from *L. peruvianum* in the asymmetric hybrids

Chromosome	Phenotypic observations				Single complementation (%)
3	*sy sf*	*sy sf*$^+$	*sy*$^+$ *sf*	*sy*$^+$ *sf*$^+$	
	3	1	0	4	12½
4	*clau di*	*clau di*$^+$	*clau*$^+$ *di*	*clau*$^+$ *di*$^+$	
	0	1	0	1	50
6	*yv c*	*yv c*$^+$	*yv*$^+$ *c*	*yv*$^+$ *c*$^+$	
	0	0	2	0	100
7	*var not*	*var not*$^+$	*var*$^+$ *not*	*var*$^+$ *not*$^+$	
	0	0	1[a]	2	33
8	*l al*	*l al*$^+$	*l*$^+$ *al*	*l*$^+$ *al*$^+$	
	0	0	0	1	0
9	*ah marm*	*ah marm*$^+$	*ah*$^+$ *marm*	*ah*$^+$ *marm*$^+$	
	0	2[b]	0	2	50
11	*hl a*	*hl a*$^+$	*hl*$^+$ *a*	*hl*$^+$ *a*$^+$	
	0	2[c]	3[d]	3	62½
	3	12		13	12/28 = 43

[a] Concerns one subclone; other subclone was *var*$^+$ *not*$^+$ (hybrid 30H36)
[b] Only sectors of one shoot; other shoots were *ah marm* (hybrid 30H36)
[c] One subclone of one hybrid; other subclone was *hl*$^+$ *a*$^+$ (hybrid 30H33)
[d] One subclone of one hybrid; other subclone was *hl a* (hybrid 30H22)

Morphological analysis

For the analysis of the retention of *L. peruvianum* alleles of morphological marker genes, we used *L. esculentum* genotypes that were homozygous recessive for such genes as the recipient (Table 1); *L. peruvianum* carries dominant alleles for all of the morphological marker genes involved. The complementation of the recessive *L. esculentum* alleles in the asymmetric 30H-hybrids is given in Table 4. In the majority of these hybrids, the recessive markers were complemented by *L. peruvianum*-specific alleles. Several hybrid calli (7 out of 97) regenerated shoots that differed with respect to the retention of *L. peruvianum* alleles.

Each of the *L. esculentum* multiple marker lines contained two markers on a single chromosome (Table 1). Therefore, it was possible to determine whether a given *L. peruvianum* chromosome was transferred completely by analysing both markers. The frequency of complementation of one or both of these genes, or of none at all, is shown in Table 5. In 43% of the plants, complementation of only one gene was observed. This suggests the presence of fragments or incomplete, deleted chromosomes of *L. peruvianum* in these asymmetric hybrids.

Discussion

The results presented in this paper show that a large amount of *L. peruvianum* genome is retained in asymmetric somatic hybrids of *L. esculentum* and irradiated *L. peruvianum*. This is in agreement with the relatively large number of donor chromosomes observed in these hybrids (Wijbrandi et al. 1990c). From our isozyme analysis of the hybrids, it appears that the high-dose hybrids (30H and 100H) retained fewer *L. peruvianum*-specific alleles than the low-dose hybrids (5H). This agrees with the observation that the high-dose hybrids resemble *L. esculentum* more than the low-dose hybrids in general morphological appearance (Wijbrandi et al. 1990c).

The limited elimination of donor genome in our asymmetric somatic hybrids is in contrast with what has been observed in asymmetric hybrids of other species, which have been shown to retain only one or a few donor chromosomes (Bates et al. 1987; Dudits et al. 1980; Gupta et al. 1984), or even one or a few traits (Dudits et al. 1987; Somers et al. 1986). This limited elimination in the tomato asymmetric hybrids could be a consequence of our unintended selection for good callus growth, since this trait is better in *L. peruvianum* than in *L. esculentum*, is multi-genic and is not linked to regeneration capacity (Koornneef et al. 1987a). Another explanation for the limited elimination could be the lack of somatic incongruity. In those hybrids where much elimination occurred relatively unrelated species (from different genera or families) had fused. No symmetric hybrids could be obtained from these species. Therefore, if somatic incongruity occurs, only hybrids with a minimal amount of donor genome can survive. When related species were fused, the elimination of the donor genome was often limited (Famelaer et al. 1989; Yamashita et al. 1989). Those species were somatic congruent since symmetric hybrids could also be obtained. Exceptions are provided by some of the asymmetric hybrids of the related species *Nicotiana tabacum* (+) *N. plumbaginifolia* (donor), which contained only one donor chromosome (Bates et al. 1987).

Kanamycin resistance was retained in 46% of the 30H-hybrids that derived from a donor parent with one NPT II locus. This percentage is an average obtained from the analysis of 16 independent kanamycin-resistant *L. peruvianum* genotypes. If the NPT II gene integrates randomly in the *L. peruvianum* genome, this would imply that any allele of the donor genome is retained in about

50% of the hybrids. In contrast with the single kanamycin resistance allele, the isozyme and morphological markers were represented by two alleles in the *L. peruvianum* donor genome. When a hybrid isozyme pattern or complementation of a mutant phenotype was observed, either one or two donor alleles were present. The average chance that a certain allele is retained, can be deduced from the number of hybrids that had lost both homologous alleles. The isozyme markers (Table 3) and morphological markers (Table 4) were distributed more or less at random over the genome. The frequency of loss of both donor alleles of a given locus in the 30H-hybrids was, on the average, 23%–31% ("mixed" is considered as lost in the latter). An individual *L. peruvianum* allele is thus lost with a frequency of $\sqrt{0.23}$–$\sqrt{0.31}$ = 48%–56%, and is therefore retained with a frequency of 44%–52%. These frequencies agree well with the frequency of retention of the kanamycin resistance alleles.

We frequently observed that shoots which derived from the same hybrid callus differed with respect to one or more of the analysed markers, namely in 2 of the 38 tested 5H-hybrids (5%), 10 of the 63 30H-hybrids (16%) and 1 of the 9 100H-hybrids (11%). Apparently, the high-dose hybrids showed this phenomenon more often. The segregation occurred most probably in the hybrid callus. Segregation at the plant level was shown in one hybrid shoot containing sectors in the leaf that were *marmorata,* the phenotype of the recipient LA1164 (Table 4). Somatic segregation has also been observed in asymmetric hybrid calli and plants of *Nicotiana plumbaginifolia* (+) (irradiated) *N. sylvestris* for several isozymes (Famelaer et al. 1989).

Because the complementation of only one of two genes on the same chromosome occurred rather frequently (Table 5), a large fraction of the asymmetric somatic hybrids must have contained incomplete chromosomes of *L. peruvianum*. In each fusion combination this could be analysed for only one or two chromosomes. Even when both markers were complemented by *L. peruvianum* alleles, this may have resulted from the retention of two incomplete chromosomes. Therefore, we conclude that the high dose of gamma irradiation (300 Gy) induced many breaks in the *L. peruvianum* chromosomes. This was confirmed by the RFLP analysis of 7 30H-hybrids, in which, on the average, at least 12 of the 18 retained donor chromosomes were incomplete (Wijbrandi et al. 1990b).

The asymmetric somatic hybrids were selected for regeneration capacity. This trait of *L. peruvianum* is supposed to be governed by two unlinked, dominant genes (Koornneef et al. 1987a). Theoretically, it should have been possible to locate these regeneration genes in our experiments due to the availability of marker genes for each of the chromosomes. Genes of *L. peruvianum* linked to the regeneration genes should be present in the asymmetric hybrids. However, we were not able to locate these genes. This could have been caused by: (1) the presence of many *L. peruvianum* chromosomes in the asymmetric hybrids; (2) the small number of asymmetric hybrids that had lost any given gene; (3) the high frequency of breakage of the donor chromosomes, so that only closely linked genes could be used for this analysis. Nevertheless, we can state that the regeneration genes are not closely linked to *sy, sf* (both mapped on chromosome 3), *hl* and *a* (both chromosome 11) because both *L. peruvianum* alleles of these genes were absent in a large fraction of the asymmetric hybrids.

Acknowledgements. We are very grateful to Prof. C. M. Rick for supplying the tomato tester lines, to Dr. P. J. M. van den Elzen for providing the plasmid pAGS112, to José Kok, Patty van Loenen Martinet-Schuringa, Anja Posthuma, René Rijken and Janny Vos for doing some of the experiments, and Prof. C. Heyting for critically reading the manuscript. This research was supported by the Foundation for Fundamental Biological Research (BION), which is subsidised by the Netherlands Organisation for Scientific Research (NWO).

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