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

1. Cytogenetics and morphology

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Summary. Asymmetric somatic hybrids of *Lycopersicon esculentum* and *Lycopersicon peruvianum* were obtained by fusion of leaf protoplasts from both species after irradiation of protoplasts or leaf tissue of *L. peruvianum* with 50, 300, or 1,000 Gy of gamma-rays. These radiation doses were sufficient to abolish the growth of the *L. peruvianum* protoplasts. The hybrids were selected for their ability to regenerate plants; this regeneration capacity derived from *L. peruvianum*. All asymmetric hybrid plants were aneuploid. The ploidy level, the morphology, and the regeneration rate were analyzed in relation to the radiation dose applied to *L. peruvianum*. After a low dose (50 Gy), most hybrids had near-triploid chromosome numbers, whereas after a high dose (300 or 1,000 Gy), most hybrids had near-pentaploid numbers. The morphology of the asymmetric hybrids was intermediate between that of *L. esculentum* and symmetric somatic hybrids of both species (obtained without irradiation treatment), and approached the morphology of *L. esculentum* to a greater extent after a high dose of irradiation. The asymmetric hybrids regenerated more slowly than the symmetric hybrids and regeneration proceeded more slowly after a high dose than after a low dose of irradiation. The high-dose hybrids also grew more slowly, flowered less, and set fruits less than the low-dose hybrids. No seeds could be obtained from any asymmetric hybrid.

Key words: Protoplast fusion – Gamma irradiation – Plant regeneration – Aneuploidy – Partial genome transfer

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Introduction

The transfer of desirable traits from wild into cultivated species is one method of improving crops. For this purpose, interspecific sexual or somatic hybrids may be constructed. The formation of sexual hybrids is limited to closely related species. Symmetric somatic hybrids, obtained by fusion of untreated protoplasts of different species, can be more easily made in some combinations. However, species hybrids, both sexual and somatic, contain many unwanted traits of the wild species besides the desired ones and are often sterile. Fertility is required to perform several backcrosses with the cultivated species to remove unwanted characters of the wild species. These problems may be circumvented by asymmetric somatic hybridization. By this procedure protoplasts of one species, the recipient, are fused with inactivated (mostly by X- or gamma-rays, protoplasts of another species, the donor. The donor genome will be fragmented and the asymmetric hybrids will contain the complete genome of the recipient species and a small part of the donor genome. The advantages of this procedure could be that hybrids arise with few unwanted donor traits, and that fewer backcrosses of the asymmetric hybrids are required to eliminate these. Several asymmetric hybrids were obtained in other studies; the fraction of donor genome that was transferred varied from one or a few traits (Dudits et al. 1987), one or a few chromosomes (Bates et al. 1987; Gupta et al. 1984) to many chromosomes (Gleba et al. 1988; Yamashita et al. 1989; Famelaer et al. 1989). Fertile asymmetric hybrids were reported in several cases and irrespective of the amount of transferred donor genome (Bates et al. 1987; Dudits et al. 1987; Gleba et al. 1988; Yamashita et al. 1989; Famelaer et al. 1989).

We are interested in the transfer of traits from the wild tomato species *Lycopersicon peruvianum* to the cul-

Table 1. The genotype of the recipient species *L. esculentum* and the irradiation treatment of the donor species *L. peruvianum*, involved in the protoplast fusion experiments. The LA-genotypes are multiple marker lines, homozygous recessive for morphological marker genes (Rick 1982b). The protoplast fusion method used is given for each combination; M = method according to Menczel et al. (1981), cms = method according to Negrutiu et al. (1986). The number of calli that did regenerate shoots is given in the far right column

Recipient	Irradiation of donor		Number of experiments	Fusion method	Regenerating calli
	Material	Dose (Gy)			
cv Bellina	protoplasts	0	1[a]	M	11
cv Bellina	protoplasts	50	3	M	53
cv Bellina	protoplasts	300	3	M	25
cv Bellina	protoplasts	1,000	3	M	13
LA291	protoplasts	300	4	M/cms	31
LA1164	protoplasts	300	1	M	16
LA1166	leaflets	300	2	M	14
LA1182	leaflets	300	1	M	8
LA1182	protoplasts	300	3	M	12
LA1189	leaflets	300	1	M	3
LA1189	protoplasts	300	2	M	7
LA1444	protoplasts	300	2	cms	9
LA1665	leaflets	300	4	M	13
LA1665	protoplasts	300	1	M	1

[a] This experiment was used to determine the regeneration frequency, as shown in Fig. 1; the recipient (ATW3003) was kanamycin-resistant to select against the nonirradiated donor species. Only a sample of 14 calli was tested on shoot induction medium

tivated tomato, *Lycopersicon esculentum*. *L. peruvianum* has many desirable characters for tomato improvement (Rick 1982a), but only a few of these (e.g., *Mi*- and TMV-resistance) have been introduced into the cultivated tomato, because sexual hybrids of both species are very difficult to obtain (Taylor 1986). In the present experiments we obtained asymmetric somatic hybrids by fusing *L. esculentum* protoplasts with irradiated *L. peruvianum* protoplasts. The hybrids were selected for the dominant regeneration capacity character of *L. peruvianum* (Koornneef et al. 1987a). Our purpose was to analyze the cytogenetics, morphology, and fertility of the regenerated hybrids in relation to the radiation dose applied to *L. peruvianum*.

Materials and methods

Plant materials

As recipient (*Lycopersicon esculentum*), the Dutch hybrid cultivar Bellina (kindly provided by Rijk Zwaan Seed Company, de Lier, The Netherlands) and the genotypes LA291, LA1164, LA1166, LA1182, LA1189, LA1444, and LA1665 from the Tomato Genetics Stock Center in Davis, USA (kindly provided by Prof. C. M. Rick) were used. The latter genotypes allow observations on the complementation of monogenic morphological mutant phenotypes by the donor (Wijbrandi et al. 1990c). Plants from the *Lycopersicon peruvianum* accession PI128650 (received from the Institute of Horticultural Plant Breeding, Wageningen, The Netherlands) were used as donor. Kanamycin-resistant plants of *L. peruvianum* and *L. esculentum* cv Bellina were also available; these had been obtained by leaf disc transformation with *Agrobacterium tumefaciens* containing the plasmid AGS112 (Koornneef et al. 1987b). Kanamycin resistance in *L. esculentum* was used to select for symmetric somatic hybrids (Wijbrandi et al. 1990a); kanamycin-resistant *L. peruvianum* was used to monitor the loss of the resistance in the asymmetric somatic hybrids (Wijbrandi et al. 1990c).

Fusion combinations

The protoplast fusion experiments are listed in Table 1. *L. peruvianum* was irradiated with gamma-rays from a ^{60}Co source at a dose rate of approximately 2,000 Gy/h at the Pilot-Plant for Food Irradiation, Wageningen (The Netherlands). Three different doses were used: 50, 300, or 1,000 Gy (= 5, 30, or 100 kRad). Putative hybrids that resulted from these fusions are designated 5H, 30H, and 100H, respectively. Symmetric hybrids, which resulted from fusions of nonirradiated protoplasts, are indicated as 0H-hybrids. The irradiation was carried out either on protoplasts, suspended at $1-3 \times 10^5$/ml in W5 solution (Menczel et al. 1981) 1–3 h before fusion, or on leaflets 1 day before fusion.

Cell culture

The isolation and culture of protoplasts are described by Wijbrandi et al. (1990a). The pretreatment of leaf material, in the dark and on pre-incubation medium, was omitted in several experiments without negative effects. Protoplast fusion was carried out either according to Negrutiu et al. (1986; the cms method) or according to Menczel et al. (1981), except that 30% PEG 4,000 was used instead of 40% PEG 6,000 (Table 1). The ratio of *L. esculentum* to *L. peruvianum* protoplasts during fusion was 1:1 to 2:1. In each fusion experiment 1×10^6 to 3×10^6 protoplasts were involved. In each experiment, cultures of the parental protoplasts, both separate and mixed, were started in parallel with the fusion cultures (i.e., cultures of mixed protoplasts of recipient and donor, after fusion treatment); in some experiments, also donor protoplasts were subjected to a fusion treatment. Rooting of regenerated shoots was induced on shoot

Table 2. Characteristics of different hybrid calli that regenerated shoots: root formation of shoots (*in vitro*), establishment in soil in the greenhouse (at least 1 month), flowering, fruit, and seed set. All hybrids were obtained after fusion of protoplasts from *L. esculentum* with protoplasts from *L. peruvianum*, which had not been irradiated with 50, 300, or 1,000 Gy of gamma-rays (5H, 30H, or 100H, respectively) before fusion

Hybrids	Regenerating calli	Root formation	Greenhouse plants	Flowers	Fruits	Seeds
0H	ND	40	32[a]	31	28	21
5H	53	38	27	21	13	0[b]
30H	139	63	32	15	5	0[c]
100H	13	9	3	1	1	0

[a] Not all rooted hybrids were transferred to soil
[b] 5 with abortive seeds
[c] 1with abortive seeds
ND – Not determined

culture medium [MS salts (Murashige and Skoog 1962), T vitamins (Tewes et al. 1984), 10 g/l sucrose], either without hormones or supplemented with 0.1 mg/l indole-butyric-acid.

The regeneration capacity of a number of asymmetric hybrids was tested as described by Koornneef et al. (1987a).

Plant characterization

The determination of chromosome numbers in root-tip cells and the assessment of several morphological characters distinguishing both species, as well as fertility, were described earlier (Wijbrandi et al. 1990a). Diploid and tetraploid plants of the parental species and symmetric somatic hybrids of both species derived from fusion experiments without radiation treatment, served as controls.

Results

Cell culture and plant regeneration

With the culture procedures employed, calli could be obtained from protoplasts of the *L. esculentum* cultivar Bellina. These calli did not regenerate shoots on shoot induction medium. The LA-genotypes of tomato differed somewhat in their protoplast culture responses. Most of them showed limited cell division and very few microcalli developed. However, growth of these *L. esculentum* calli seemed to be improved in cultures where they were mixed with irradiated *L. peruvianum* protoplasts, which apparently behaved as feeder cells. In the case of LA1182, a tetraploid plant with the LA1182 phenotype could be regenerated from such a mixed culture.

The *L. peruvianum* protoplasts that were irradiated with 50, 300, or 1,000 Gy of gamma-rays formed cell walls. Some of these cells strongly increased in size, while others divided once or twice. In 1 of 12 experiments where *L. peruvianum* protoplasts, irradiated with 300 Gy, were subjected to a fusion treatment, we obtained three calli; these did not regenerate plants.

The fusion cultures yielded many calli, of which several formed shoots. Within each experiment there was a large variation in callus as well as shoot morphology.

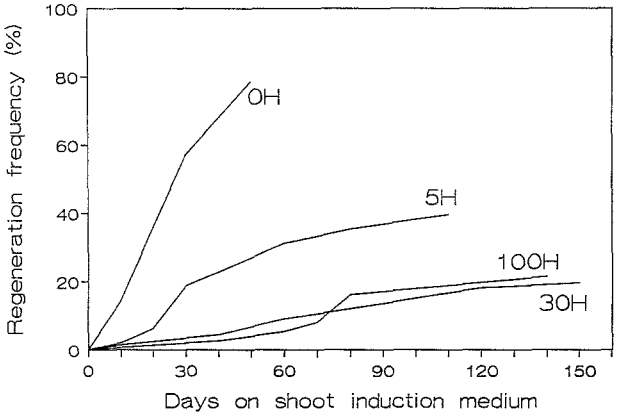

Fig. 1. Regenerated frequency, i.e., the percentage of calli tested that formed shoots on shoot induction medium. The calli were derived from cultures of mixed, fusion-treated protoplasts of *L. esculentum* cv Bellina and *L. peruvianum* PI128650-ATW2002, which latter were irradiated with 50, 300, or 1,000 Gy of gamma-rays (5H, 30H, 100H; 48, 66, 37 calli tested, respectively) and of *L. esculentum* cv Bellina-ATW3003 and nonirradiated *L. peruvianum* PI128650 (0H); 14 calli tested). The 0H-hybrid calli derived from a fusion experiment of a kanamycin-resistant Bellina and a *L. peruvianum* sensitive to this antibiotic, so that selection against the *L. peruvianum* parent was possible

Figure 1 shows the time course of shoot formation by asymmetric hybrid calli, resulting from fusions where different radiation doses were applied to the donor protoplasts. The rate of shoot regeneration as well as the fraction of calli that could form shoots decreased with the radiation dose. The regeneration efficiency of the symmetric somatic hybrids (0H in Fig. 1) is similar to that of nonirradiated *L. peruvianum* (data not shown).

The characteristics of the putative hybrid calli that regenerated shoots with respect to root formation, establishment in soil, flowering, and seed set are shown in Table 2 for each irradiation dose. The 5H-hybrids could be transferred to soil more efficiently than the 30H- and 100H-hybrids.

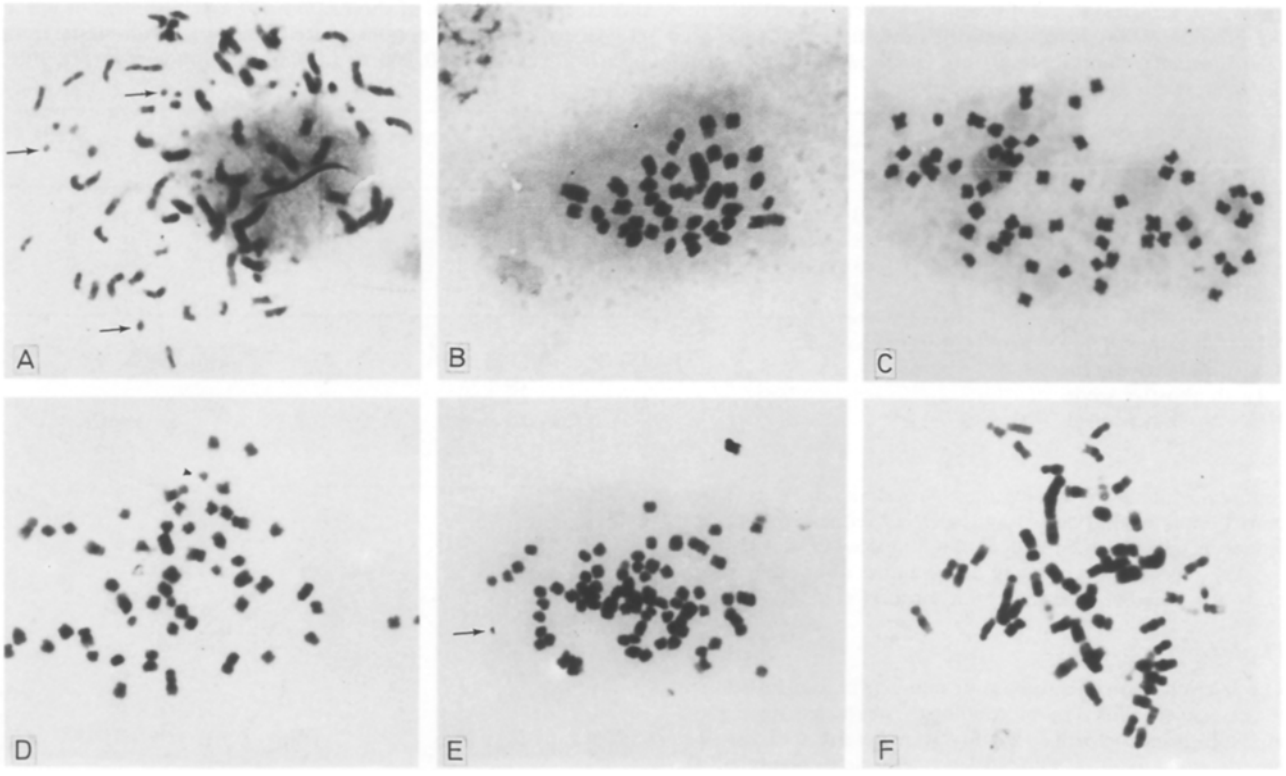

Fig. 2A–F. Metaphase plates of root-tip cells from asymmetric somatic hybrids of *L. esculentum* and irradiated *L. peruvianum*. **A** 5H24 (radiation dose of 50 Gy was applied), 2n = about 66. **B** 5H15, 2n = 43. **C** 5H19, 2n = 57. **D** 30H46 (dose of 300 Gy), 2n = 56. **E** 30H28, 2n = 70. **F** 100H3 (dose of 1,000 Gy), 2n = 57. Notice the small particles which probably are centric chromosome fragments (*arrows*)

Table 3. Regeneration capacity, measured as shoot formation on shoot induction medium of established callus cultures, which were induced on leaf explants of *L. esculentum*, *L. peruvianum*, and somatic hybrids. The number of independent hybrid calli, from which the tested plants derived, is given between parentheses. The hybrids were obtained after fusion of *L. esculentum* protoplasts with *L. peruvianum* protoplasts, which had not been irradiated (0H) or irradiated with 50, 300, or 1,000 Gy of gamma-rays (5H, 30H, or 100H, respectively) before fusion

Plants	Regeneration capacity	
	+	−
L. esculentum	0	4
L. peruvianum	7	0
0H-hybrids	2 (2)	0
5H-hybrids	14 (9)	1 (1)
30H-hybrids	6 (5)	3 (3)
100H-hybrids	1 (1)	0

+, callus regenerating; −, callus not regenerating

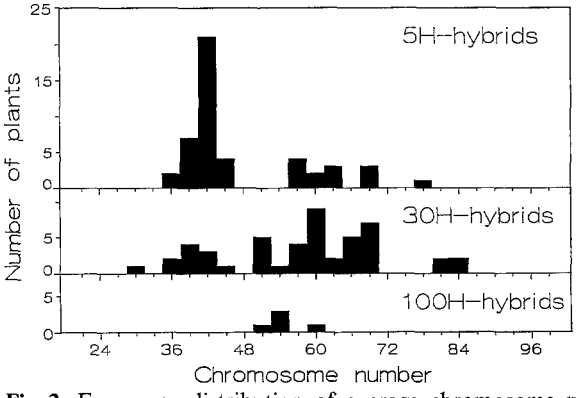

Fig. 3. Frequency distribution of average chromosome numbers of different shoots from asymmetric somatic hybrids of *L. esculentum* and *L. peruvianum* (the donor species). *Upper panel*: 5H-hybrids (donor irradiated with 50 Gy); *middle panel*: 30H-hybrids (300 Gy); *lower panel*: 100H-hybrids (1,000 Gy)

Only 1 of 15 5H-hybrid plants tested could not regenerate shoots from established callus (Table 3), whereas 3 of 9 30H-hybrids did not. The controls reacted as expected (Koornneef et al. 1987a): *L. esculentum* was nonresponsive, whereas all tested *L. peruvianum* plants and 0H-hybrids showed plant regeneration from established callus cultures.

Cytogenetics

The parental species both have 2n = 2x = 24, relatively small chromosomes. The *L. peruvianum* chromosomes are homoeologous to the *L. esculentum* chromosomes

Fig. 4. The leaves of 12 different asymmetric somatic hybrids, *L. esculentum* cv Bellina (*left*) and *L. peruvianum* PI128650 (*right*). All hybrids were obtained after protoplast fusion of *L. esculentum* cv Bellina with *L. peruvianum*, which was irradiated with 50 Gy of gamma-rays before fusion

(Rick and Butler 1956). Most of the symmetric somatic hybrids between these species had a chromosome number of 48 or 72 (2n = 4x and 6x, respectively; Wijbrandi et al. 1990a). The chromosome numbers of the asymmetric somatic hybrids varied from 29 to 85, as presented in Figs. 2 and 3. The majority of the 5H-hybrids had chromosome numbers below the tetraploid level (shoots from 13 of 21 hybrid calli), whereas the 30H- and the 100H-hybrids usually were above the tetraploid level (shoots from 17 of 25 30H-calli and both 100H-calli), A few hybrids even exceeded the hexaploid level (shoots from 1 5H- and 3 30H-hybrid calli). The counting of the chromosomes was hampered by their small size. In several cases very small particles, most probably centric chromosome fragments, were observed (Fig. 2). In general, the chromosome number of each shoot varied slightly within and between the analyzed roots. In a few cases, larger differences were observed, mostly occurring between different roots: the chromosome numbers differed by 10 to 20. Normally the shoots from one fusion callus had similar chromosome numbers. Also, when shoots of different subclones of the same hybrid callus were present, they mostly (4 of 5 calli) had similar chromosome numbers. However, one hybrid, 30H28, deviated: four healthy-looking shoots from one subclone had 41 and a fifth had 70 chromosomes, whereas the only shoot from the other subclone probably had 29 chromosomes (only two cells counted); the shoots with 29 and 70 chromosomes grew more poorly than the four other shoots derived from the same callus.

Plant morphology

The asymmetric hybrid plants were less vigorous than both parental species and the symmetric hybrids. The 30H- and 100H-hybrids in particular were very retarded in growth and often died after transfer to soil. The leaves of the asymmetric hybrids differed greatly in color (from light- to grey-green), shape, and size (Fig. 4). Generally the leaves were smaller than those of the parental species (Fig. 5A). Leaves of the 30H- and 100H-hybrids were mostly smaller than those of the 5H-hybrids. Usually the asymmetric hybrids had leaves with deep incisions, similar to *L. esculentum,* and secondary leaflets, like *L. esculentum* and the symmetric hybrids. No stipules were present on the stems of the 30H-hybrids and only a few small ones were on the stems of the 5H-hybrids (Fig. 5B); the same holds for the presence of bracts (leaflets in the inflorescences; Fig. 5C). The sympodial index (i.e., the mean number of nodes between two subsequent inflores-

cences) of the asymmetric hybrids in general was three, similar to that of the cultivated tomato and the symmetric hybrids.

Flower morphology

A majority of the greenhouse-grown 5H-hybrids and a minority of the greenhouse-grown 30H- and 100H-hybrids formed flowers (Table 2). The asymmetric hybrids flowered less abundantly than the parents and the symmetric hybrids. The corollas of the 5H-hybrid flowers were in general dark yellow, like those of *L. peruvianum* and the symmetric hybrids, whereas most 30H-hybrids and the only flowering 100H-hybrid had pale yellow flowers, similar to the *L. esculentum* ones. The size of the flower parts as well as the ratios of the sizes of different flower parts varied strongly between hybrids (Fig. 6A and 6B). The average size of the flowers of the 5H-hybrids was similar to that of the parental species, whereas the 30H-hybrids had smaller flowers. The relative sepal length of the asymmetric hybrids usually was intermediate between *L. esculentum* and the symmetric hybrids. In most plants of both classes of asymmetric hybrids the pistil was smaller than the stamens; this was also observed in tetraploid *L. esculentum* plants (Wijbrandi et al. 1990a).

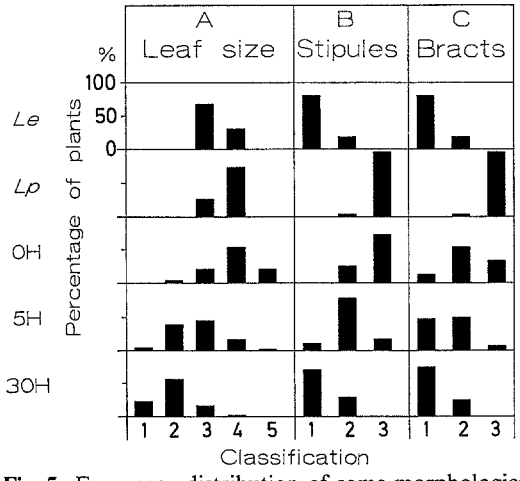

Fig. 5. Frequency distribution of some morphological characters of *L. esculentum* (*Le*; 16 plants tested), *L. peruvianum* (*Lp*; 22 tested), symmetric somatic hybrids of both species (0H; 60 tested), and asymmetric somatic hybrids of both species, in which occasion *L. peruvianum* was irradiated before fusion with 50 and 300 Gy of gamma-rays (5H and 30H; 41 and 34 shoots tested, respectively). *A* Leaf size: an arbitrary classification. 1 = very small leaves (< 2 cm) to 5 = very large leaves (> 50 cm). *B* Presence of stipules: leaflets on the stem at the nodes. *C* Presence of bracts: leaflets in the inflorescence. Classes of B + C: 1 = absence; 2 = presence at a few nodes; 3 = presence at each node

Table 4. The color of the fruits obtained from different asymmetric somatic hybrids were obtained after protoplast fusion of *L. esculentum* with *L. peruvianum*, which was irradiated with 50, 300, or 1,000 Gy of gamma-rays (5H, 30H, or 100H, respectively) before fusion. Each observation was done on one to several shoots from one hybrid callus

Plants	Fruit color		
	Yellow	Orange	Red
5H-hybrids	5	6	1
30H-hybrids	0	4	1
100H-hybrids	0	1	0

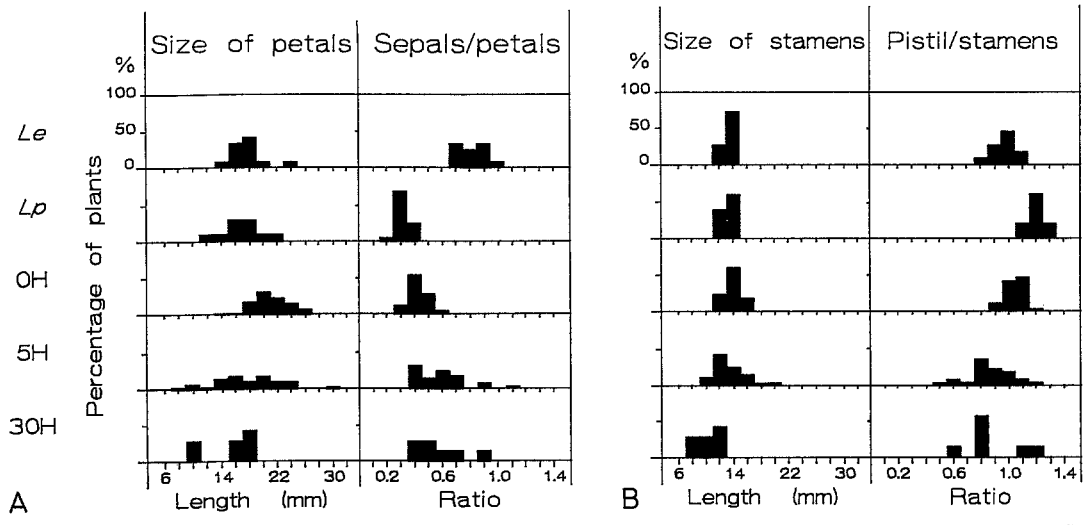

Fig. 6A and B. Frequency distribution of some sizes of flower parts of *L. esculentum* (*Le*; 12 plants tested), *L. peruvianum* (*Lp*; 20 tested), symmetric somatic hybrids of both species (0H; 39 tested), and asymmetric somatic hybrids of both species, in which occasion *L. peruvianum* was irradiated before fusion with 50 and 300 Gy of gamma-rays (5H and 30H; 28 and 7 shoots tested, respectively). **A** Size of the petals and relative length of the sepals (with respect to the petal length). **B** Size of the stamens and relative length of the pistil (with respect to the stamen length). Each measurement was the average of at least two flowers from the same plant

Fertility

The flowers of the asymmetric somatic hybrids produced no or small amounts of pollen that were not viable, as tested with lactophenol acid fuchsin staining.

Several asymmetric hybrids set fruits (Table 2), measuring in diameter from 0.5 to a few centimeters. Usually they were smaller than *L. peruvianum* fruits (1–2 cm) and much smaller than tomato fruits (>3 cm). Also the color of the fruits differed between hybrids (Table 4). Most fruits were orange, which is intermediate between the fruit color of the symmetric hybrids (yellow) and the cultivated tomato (red).

No seed set was obtained in the asymmetric hybrids after selfing or backcrossing with the parental species (Table 2); the symmetric hybrids, on the other hand, did set seeds. In some fruits of five 5H-hybrids and one 30H-hybrid, very small, abortive seeds were observed.

Discussion

The present experiments show that asymmetric somatic hybrid plants can be derived from protoplast fusions between *L. esculentum* and irradiated *L. peruvianum*. Selection of these hybrids was possible on the basis of the regeneration capacity derived from the irradiated species. The asymmetric hybrid nature could be confirmed by several morphological characteristics, which were intermediate between those of the symmetric somatic hybrids and those of *L. esculentum*, and by the analysis of specific marker genes (Wijbrandi et al. 1990c). However, the irradiation of *L. peruvianum* before fusion did not have the desired effect, namely, elimination of the donor genome to such an extent that only a small fraction was conserved in the asymmetric hybrids.

All asymmetric hybrids were aneuploid. From the cytogenetic and morphological analysis, we tentatively conclude that most asymmetric hybrids contained one, two, or three diploid *L. esculentum* genomes, supplemented with one partial diploid genome of *L. peruvianum*. For 15 asymmetric hybrids this was confirmed by RFLP analysis (Wijbrandi et al. 1990b). The 5H-hybrids, of which most had one diploid *L. esculentum* genome, had on the average 16 presumed *L. peruvianum* chromosomes (range: 5–22); the 30H-hybrids, which in most cases probably contained a tetraploid genome of *L. esculentum*, had a mean of 13 (range: 2–22) *L. peruvianum* chromosomes; and both 100H-hybrids probably had a tetraploid *L. esculentum* genome and, on the average, 7 (range: 4–12) *L. peruvianum* chromosomes. Although there is no clear correlation between the radiation dose and elimination of the *L. peruvianum* genome, the 30H-hybrids resembled *L. esculentum* more than the 5H-hybrids. This is not necessarily due to the elimination of *L. peruvianum* chromosomes, but can also be ascribed to the presence of a larger number of *L. esculentum* genomes in most 30H-hybrids. In any case, a relatively large fraction of the *L. peruvianum* genome is retained, even in the 100H-hybrids. Limited chromosome elimination, together with a weak dose effect, was also observed in asymmetric somatic hybrids of other species. Retention of the donor chromosomes ranged from 11% to 90% (of a diploid donor genome) in *Nicotiana plumbaginifolia* (recipient) (+) *Atropa belladonna* (donor) hybrids (100–1,000 Gy; Gleba et al. 1988), from 8% to 75% in *N. plumbaginifolia* (+) *N. sylvestris* hybrids (100–1,000 Gy; Famelaer et al. 1989), and from 25% to 100% in *Brassica oleracea* (+) *B. campestris* hybrids (100–800 Gy; Yamashita et al. 1989). In all those cases, chromosome rearrangements and/or deleted donor chromosomes were reported. In some of the *L. esculentum* (+) *L. peruvianum* asymmetric hybrids reported here, we observed fragments (Fig. 2). Such small fragments were also observed in somatic hybrid plants of potato (Pijnacker et al. 1989), and the very tiny ones resemble "double minutes," which can be found in mammalian cancer cells (Cowell 1982). Moreover, the RFLP analysis, applied to 15 of the asymmetric hybrids, clearly indicated the presence of incomplete chromosomes in all tested plants (Wijbrandi et al. 1990b).

The asymmetric 5H-hybrids were more viable than the 30H- and 100H-hybrids, irrespective of whether shoot regeneration, root formation, or morphological characteristics were used as criteria, whereas all asymmetric hybrids were less viable than the symmetric hybrids. A possible explanation for this is the unbalanced genome of the asymmetric hybrids. It has been reported that in *L. esculentum* only primary trisomics and some monosomics are viable (Rick and Butler 1956), whereas aneuploidy is tolerated better by primitive tomatoes and species hybrids of the tomato than by the cultivated tomato (Soost 1958; Rick and Notani 1961; Györffy and Mako 1963). In general the asymmetric somatic hybrids were near the triploid or pentaploid level; the 5H-hybrids usually had a diploid *L. esculentum* genome, whereas most 30H- and 100H-hybrids presumably had a tetraploid recipient genome. In other asymmetric hybridization experiments that resulted in limited chromosome elimination (Gleba et al. 1988; Famelaer et al. 1989; Yamashita et al. 1989), the recipient genome in the asymmetric hybrids usually was around tetraploid or hexaploid. An explanation of this could be that cells with polyploidized recipient genomes, which may have arisen during the tissue culture phase, better tolerate additional and abnormal chromosomes (rearrangements and fragments).

The variation in chromosome number with in each of the asymmetric hybrids might indicate instability at the plant level. Rearranged and incomplete chromosomes

probably tend to get lost more frequently. The observed loss of regeneration capacity in established callus cultures of some 30H-hybrids might be explained this way. Another explanation is elimination of this trait in the established callus cultures.

The limited elimination of donor chromosomes is a serious drawback for the application of asymmetric hybrids in plant breeding, because several backcrosses to the recurrent parent are still required to eliminate the unwanted donor traits. Crosses will be impossible or at least hampered strongly by the sterility and the polyploidy of these hybrids. The sterility is probably due to the cytogenetic aberrations, such as aneuploidy and rearrangements. The latter were more frequent after higher radiation doses (Wijbrandi et al. 1990b). Despite the fact that several scores of asymmetric hybrids were obtained and analyzed, none of the plants had just one or two chromosomes above the diploid level, which probably would have allowed backcrossing to the diploid recipient. If the polygenic nature of the selectable donor traits (both callus growth and regeneration characteristics) is the main cause of this limited elimination, the use of simpler selectable markers, such as antibiotic resistance or alleles complementing auxotrophic mutations, would help to overcome the problem. If not, other ways to enhance elimination of donor chromosomes should be sought or very large populations of asymmetric hybrids must be evaluated.

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