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Sexual and somatic hybridization in the genus *Lycopersicon*

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Abstract. In recent years, a large number of reports have been published on the recovery of somatic hybrids in the genus *Lycopersicon* and their potential use as a tool in plant breeding programs. Somatic hybridization as a way of enabling the incompatibility barriers which exist within the genus *Lycopersicon* to be bypassed has attracted great interest. Wild *Lycopersicon* species harbor numerous interesting agronomic characteristics, which could be transferred to tomato by somatic hybridization. In particular, the production of asymmetric hybrids is explored as an approach to obtain the transfer of only a part of the nuclear genome of wild *Lycopersicon* species. Considerable information is available on the fate of chloroplasts and mitochondria in fusion products in *Lycopersicon*, and unfortunately, cybridization (transfer of chloroplasts and/or mitochondria) seems often difficult to achieve.

Key words: *Lycopersicon* – Sexual hybridization – Somatic hybridization – Cybridization

1 Introduction

Tomato (*Lycopersicon esculentum*) is an important food crop; world production in 1989 was 63,316 Mtons (Kalloo 1991). Several countries produce high yields of tomatoes for use directly as a vegetable and also to prepare paste, juice and ketchup. The improvement of tomato started several centuries ago in western South America, its area of natural distribution. It was subsequently introduced into different countries, and numerous varieties with interesting agronomic characteristics have been developed (Rick 1983; Philouze 1986). The genus *Lycopersicon* comprises nine different species, eight of which are strictly wild, but they are important sources of agronomic quality (Rick et al. 1987; Daunay et al. 1991). Sexual crosses between these species and tomato are limited due to bilateral and unilateral incompatibility (Hogenboom 1972). Techniques to overcome crossability problems and to introgress "wild" genes into tomato have been described (Thomas and Pratt 1981; Gradziel and Robinson 1989a, b), but crosses involving certain wild species as the pistillate parent always fail. Therefore, sexual hybrids always carry the tomato organellar genomes: tomato is a plant in which existing data indicate that plastids are inherited in a strictly uniparental-maternal fashion (Smith 1989). The induced fusion of isolated protoplasts followed by the regeneration of plants constitutes a way of circumventing sexual crossing barriers and provides new approaches to genetic manipulation (Shepard et al. 1983; Kumar and Cocking 1987). This fusion can be achieved by: (1) the combination of two complete genomes; (2) partial genome transfer from a donor to a recipient protoplast; (3) the transfer of organelles (chloroplasts and mitochondria) in relation to properties such as herbicide resistance and cytoplasmic male sterility. Protoplast isolation from leaves was developed in the 1960s (for review see Chupeau and Bourgin 1980). More recently, protoplasts have been induced to fuse under controlled and repeatable conditions to give heterocaryons (Kao and Michayluk 1974). Chemical methods of plant protoplast fusion were developed in the early 1970s, and electric fields were used in the 1980s (Jones 1988). The resulting hybrid cells were cultured and the generation of intact plants induced. The genotypic nature of the somatic hybrid plants regenerated from heterocaryons largely depends on the extent of genomic diver-

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gence between the fusion partners and the application of a selection pressure in relation to the expression of marker genes in the donor plants.

Interspecific and intergeneric somatic hybridizations involving tomato have been achieved by several research groups (Melchers et al. 1978; O'Connell and Hanson 1986; Wijbrandi 1989). The use of new techniques of biochemistry and molecular biology has contributed to the determination of the genotypic nature of the somatic hybrid plants. We believed that it would be interesting to put together the results of the different experiments and study the effects of fusion in the case of *Lycopersicon* protoplasts. At the present time, the most promising practical application seems to be the exchange of useful agronomic traits between two sexually incompatible genera/species.

2 Sexual hybridization in *Lycopersicon*

2.1 The genus Lycopersicon

The group of species that includes the tomato was originally regarded as a part of the genus *Solanum*. It was not until 1754 that Miller proposed a separate genus – *Lycopersicon* (Wann and Johnson 1963) – which consists of nine species: *L. esculentum* is the cultivated species, and the others are strictly wild. A key for the *Lycopersicon* species has been formulated (Rick et al. 1990b) and by this means it is easy to establish a simplified classification of the *Lycopersicon* species (Table 1).

In 1951 Rick reported the successful hybridization of *L. esculentum* and *S. lycopersicoides*, and demonstrated that the genus *Lycopersicon* is related in particular to the section Tuberarium, series Juglandifolia of the genus *Solanum*, which comprises the species *S. lycopersicoides*, *S. juglandifolium*, *S. ochrantum* and *S. rickii*.

Table 1. A simplified classification of the *Lycopersicon* species (Rick et al. 1990b)

Subgenus *Eulycopersicon:* red- and orange-fruited, self-compatible species:
- *L. esculentum* Mill.
- *L. esculentum* var *cerasiforme* (Dun.) Gray
- *L. pimpinellifolium* (Jusl) Mill.
- *L. cheesmanii* Riley

Subgenus *Eriopersicon:* green-fruited and mostly self-incompatible species:
- *L. hirsutum* Humb. and Bonpl.

Subgeneric "minutum" complex
- *L. parviflorum* Rick, Kesicki, Fobes and Holle
- *L. chmielewskii* Rick, Kesicki, Fobes and Holle

Subgeneric *peruvianum* complex
- *L. chilense* Dun.
- *L. peruvianum* (L.) Mill.
- *L. pennellii* (Corr.) d'Arcy

2.2 Intra- and interspecific incompatibility reactions

Although there is a close relationship among the species of *Lycopersicon* – they all have 12 pairs of chromosomes, important breeding barriers do exist between them. In 1972, a brief survey of these intra- and interspecific breeding barriers was given by Hogenboom (Table 2). Sometimes barriers between species only occur in part of the material.

L. esculentum, *L. pimpinellifolium* and *L. cheesmanii* are self-compatible species, while the others are mostly self-incompatible (SI). The self-incompatibility of the *Solanaceae* is gametophytic (i.e. when an allele in the individual haploid pollen grain is matched with either allele in the diploid style tissue, growth of the pollen tube is arrested) and governed by a single S-gene with multiple alleles (Ebert et al. 1989). In *L. peruvianum*, S-allele specificity is determined by a single locus that has been mapped to chromosome 1 (Tanksley and Loaiza-Figueroa 1985). In *L. peruvianum* and in another member of the *Solanaceae* (*Nicotiana alata*), S-glycoproteins have been shown to have RNase activity (Mau et al. 1986; McClure et al. 1989). McClure (1990) have also reported that pollen RNA becomes degraded after incompatible pollination but not after compatible pollinations. Numerous studies on the genetic control of SI in *L. peruvianum* have been carried out (de Nettancourt et al. 1971; Hogenboom 1972; de Nettancourt et al. 1974; Maheswaran et al. 1986). In the 1960s, breaking this barrier appeared to be very important as it was assumed that the S-gene was involved in unilateral incompatibility (Abdal-

Table 2. Intra- and interspecific breeding barriers between the *Lycopersicon* species (from Hogenboom 1972)

Female \ Male	*L. esc.*	*L. pimp.*	*L. che.*	*L. min.*	*L. hirs.*	*L. peru.*	*L. chil.*	*L. pen.*
L. esc.	+	+	+	+	+	EA	EA	+
L. pimp.	+	+	?	+	+	EA	EA	?
L. che.	+	?	+	?	?	?	?	?
L. min.	+, UI, EA	+, UI	?	SI	EA	EA	EA	?
L. hirs.	+, UI	+, UI	?	+, UI	+, SI, UI	EA	EA	?
L. peru.	UI	UI	+	UI	UI	SI	EA	+
L. chil.	UI	UI	+	UI	?	EA	SI	?
L. pen.	UI	?	+	?	?	?	?	SI

+, No serious barrier; SI, self-incompatibility; UI, unilateral incompatibility; EA, embryo abortion; ?, no research results known

la and Hermsen 1972). Unilateral incompatibility (UI) occurs when self-compatible species are used as the pollen parent in crosses with self-incompatible species. Several hypotheses have been elaborated by different authors to account for UI, and some authors have assumed that UI is controlled by self-incompatibility alleles with dual function. First, Martin presented his hypothesis of a polygenically controlled balance affecting pollen-tube growth and stylar inhibition to account for unilateral relations (Martin 1964, 1967, 1968). In 1973 Hogenboom demonstrated that in relationships between partners from different populations, the non-functioning of a partner relationship can result from a lack of genetic information in one partner about the other (due to evolutionary divergence). This phenomenon was named "incongruity", and it was mentioned that self-incompatibility probably plays little or no role in this phenomenon. In 1991 studies of the expression of unilateral incompatibility were carried out with pollen of *L. pennellii* (Chetelat and De Verna 1991) and it was observed that major loci on chromosomes 1, 6 and 10 were involved. The results were discussed in relation to existing genetic models for unilateral incompatibility, including the possible involvement of the S-locus.

3 Sexual hybrids

3.1 Interspecific crosses

In recent years tomato breeders have concentrated on obtaining increased yields, improved fruit quality (Stevens 1986), altered plant growth (Stevens 1986) and disease and pest resistance (Rick et al. 1987; Daunay et al. 1991; Kalloo 1991). The wild *Lycopersicon* species are rich sources of germplasm for tomato improvement (Rick et al. 1987; Patterson 1988; Laterrot 1989), and the genus *Lycopersicon* is well suited as an object of investigation in the production of interspecific sexual hybrids: numerous monogenic markers are known for *L. esculentum* (Tanksley and Mutschler 1990), and hybridization between different species is not complicated by differences in chromosome number ($2x = 2n = 24$).

If tomato is used as the female parent, it is easy to obtain F_1 hybrids by crosses between tomato and the wild species *L. pimpinellifolium*, *L. cheesmanii*, *L. parviflorum*, *L. chmielewskii* and *L. pennellii*. F_1 hybrids between tomato (female parent) and *L. hirsutum* are also easy to produce; these plants are often sterile but could be backcrossed with *L. esculentum* (used as female parent). It is more difficult to obtain interspecific (*L. esculentum* × *L. chilense*) and (*L. esculentum* × *L. peruvianum*) hybrids because the embryos abort in the seed during fruit ripening (Barbano and Topoleski 1984): the events from pollen-tube growth to fertilization are normal, but post-zygotic congruity appears to be rare. Ehlenfeldt and Hanneman Jr (1992) recently carried out a study to evaluate the crossability relationships between *Lycopersicon* species in light of the EBN (endosperm balance number). Their results support the concept of two intra-fertile *Lycopersicon* groups; the "*esculentum* group" and the "*peruvianum* group". However, Bohn (1948) indicated that *L. esculentum* and *L. peruvianum* are inter-crossable if $4 \times$ *L. esculentum* is used as the female and $2 \times$ *L. peruvianum* as the male. Different techniques have been used to obtain hybrid plants between *L. esculentum* and *L. chilense* or *L. peruvianum*: embryo culture (Smith 1944), embryo callus culture (Thomas and Pratt 1981), ovule culture (Imanishi 1988), use of the immunosuppressant cuprenil, hormonal treatments and bud pollination (Gradziel and Robinson 1991). F_1 hybrids are often sterile (Lesley 1950; Rick 1963), but these techniques can effectively allow the development of bridge lines possessing "high crossability" and the ability to be backcrossed with *L. esculentum* (Poysa 1990; Gradziel and Robinson 1991).

Several "wild" genes have been integrated into tomato. The most widely used are the disease and pest resistance genes (for review Kalloo 1991; Laterrot 1989). Some tomato varieties also carry new morphological, physiological and biochemical characteristics that originate from wild *Lycopersicon* species (the "jointless" gene from *L. chersmanii* and the high soluble solids content from *L. pimpinellifolium*).

Tomato is a species that is often grown under sub-optimal conditions, and the development of cultivars possessing resistance to stresses (cold, heat, drought, excessive moisture and salinity) is now being attempted. The capacity of wild *Lycopersicon* species to survive in situations of environmental stress has been outlined (Rick 1982), however, the genetic control of these characteristics is difficult to study due to their high environmental variance and to the epistasis that occurs between the introduced genes and the *esculentum* genetic background (Guy 1990).

3.2 Intergeneric hybrids

L. esculentum has been crossed with those *Solanum* species (series Juglandifolia) that bear the closest morphological resemblance to *Lycopersicon* (Wann and Johnson 1963). Like *Lycopersicon* species, *Solanum* species have 12 pairs of chromosomes. F_1 hybrids have been obtained between tomato and *S. lycopersicoides*, but they were sterile and could not be backcrossed with tomato (Menzel 1962). In 1987 de Verna et al. described the recovery of partially fertile sesquidiploids from the backcross of the tetraploid hybrid to diploid *L. esculentum*. In 1989 Gradziel and Robinson introgressed genes from *S. lycopersicoides* into tomato through the systematic avoidance and suppression of breeding barriers. The other species

of the Juglandifolia series (*S. juglandifolium*, *S. ochrantum* and *S. rickii*) are known to have high levels of arthropod resistance and some other interesting characteristics (Rick et al. 1987), and attempts are being made to introgress germplasm from these species into tomato (De Verna et al. 1990; Rick et al. 1990a).

3.3 Potential for sexual hybridization

Genes from *L. chilense*, *L. peruvianum* and a number of other wild *Lycopersicon* species have been introgressed into tomato. The transfer of organelles of wild *Lycopersicon* species into tomato has also been obtained-twice: Mutschler (1990) created a plant containing more than 99% of the tomato genome and the *pennellii* cytoplasm, and Gradziel and Robinson (1991) obtained plants that possess approximately one-quarter of the *L. esculentum* genome and a *L. peruvianum* cytoplasm. However, these programs were long and difficult to carry out, and an efficient method for routinely transferring genes (and organelles) from wild *Lycopersicon* species into tomato has not yet been established.

4. *Lycopersicon* cell culture

4.1 Regeneration ability of Lycopersicon protoplasts

The morphogenetic responses of leaf callus from *L. esculentum* has been investigated in detail (Frankenberger et al. 1981; Zelcer et al. 1984; Uddin et al. 1988). Shoot regeneration efficiency is influenced by the genotype (Zelcer et al. 1984) and the type and concentration of the growth regulators in the culture medium (Uddin et al. 1988). The influence of the developmental maturation state of the tissue used as the source of explants has also been studied (Frankenberger et al. 1981). In contrast, little is known about the conditions allowing the sustained division and regeneration of tomato cultured protoplasts. These are obviously of great interest (Hille et al. 1989), and several authors have shown that tomato mesophyll protoplasts can be regenerated into plants (Koblitz and Koblitz 1982; Morgan and Cocking 1982; Niedz et al. 1985; Attathom and Visessuwan 1990). Several factors seem to be important for the viability of tomato protoplasts (Bellini et al. 1990) and for plant regeneration (Niedz et al. 1985), but results are difficult to obtain in a consistent manner. It has been suggested that the growth conditions of the donor plants are critical to the viability and growth of the protoplasts (Niedz and Sink 1988). The study of factors such as plant and leaf age, as well as light intensity and photoperiod during growth of the donor plants, have been neglected, but it is probably of importance to establish an efficient procedure (Shahin 1985). Compared to *L. esculentum*, the wild *Lycopersicon* species are much easier to regenerate from protoplasts (Hassanpour-Estahbanati and Demarly 1985a, b; Montagno et al. 1991; Lefrançois and Chupeau 1993: Their protoplasts divide, grow actively in different liquid media and consistently regenerate shoots. Wild *Solanum* species are also easy to regenerate (Tan et al. 1987a). Tomato genotypes with a superior ability to regenerate plants from protoplasts have been obtained by transferring regeneration capacity from *L. peruvianum* (Koornneef et al. 1987) and from *S. lycopersicoides* (Gleddie et al. 1989) into *L. esculentum* by classical breeding methods. The favorable cell culture traits of *L. peruvianum* are dominant (Koornneef et al. 1987), and a *L. peruvianum* gene controlling shoot regeneration in tomato has been mapped on chromosome 3 (Koornneef et al. 1993).

4.2 Isolation of selectable cell markers

Different kinds of markers are available in tomato. Some are expressed at the cell level and would thereby enable the development of a selection strategy of the tomato cell in somatic hybridization experiments. Dominant selectable markers, such as resistance to the antibiotic kanamycin, have been introduced into tomato by genetic transformation (Koornneef et al. 1987). Following the expression of antibiotic resistance genes, tomato cells and heterocaryons can be selected for their ability to grow in the presence of the antibiotic. Mutant plants also provide a source of selectable cell markers. A variant cell clone of *L. pennellii* resistant to G418 has been isolated by screening (Adams and Quiros 1985). Nitrate reductase deficient (NAR) mutants of tomato have been isolated by Schoenmakers et al. (1991). NAR mutants are resistant to chlorate, and they cannot grow on medium containing nitrate as the sole nitrogen source. This means that, depending on the growth medium used, either the mutant of the fusion partner can be selected.

For the analysis of organelle transfer and interaction in cybrids, the presence of selectable and easily screenable organelle genetic markers is an advantage. Several experiments of mutagenesis in tomato and other *Lycopersicon* species have produced plastid mutations and furnished selectable plastid markers. Hosticka and Hanson (1984) have treated tomato seeds with nitromethylurea and have obtained variegated plants. The chlorophyll-deficient mutations are cytoplasmically inherited. A similar system of mutagenesis has been used by McCabe et al. (1989) for selection of streptomycin-resistant mutants in *Solanaceae*, but there is evidence for Mendelian inheritance of the resistance in *L. peruvianum*. Streptomycin resistance in *L. peruvianum* has also been induced by Jansen et al. (1990), and this streptomycin resistance seems to be a chloroplast-encoded mutation. In 1990 Glas et al. isolated lincomycin-resistant plants of *L. peruvianum* using nitromethylurea mutagenesis, and crosses

Table 3. Somatic hybridization experiments involving *Lycopersicon* species

Fusion partners		Authors	RC[b]	GS[c]
L. peruvianum (lvs)[a]	*L. pennellii* (scc)	Adams and Quiros (1985)	+	−
L. peruvianum (scc)	*L. esculentum* (lvs)	Kinsara et al. (1986)	+	+
L. chilense (scc)	*L. esculentum* (lvs)	Bonnema and O'Connell (1992)	+	−
L. pennellii (c)	*L. esculentum* (lvs)	O'Connell and Hanson (1987)	+	−
S. lycopersicoides (c)	*L. esc.* × *L. pennellii* (lvs)	Guri et al. (1991)	+	−
S. lycopersicoides (scc)	*L. esculentum* (lvs)	Handley et al. (1986)	+	−
S. nigrum (lvs)	*L. esculentum* (lvs)	Guri et al. (1988)	+	−
S. rickii (scc)	*L. esculentum* (lvs)	O'Connell and Hanson (1986)	+	?
S. acaule (?)	*L. esculentum* (?)	Schweizer et al. (1988)	+	?
S. muricatum (lvs)	*L. esculentum* (lvs)	Sakomoto and Taguchi (1991)	+	−
S. tuberosum (?)	*L. pimpinellifolium* (?)	Okamura (1987)	+	?
S. tuberosum (c)	*L. esculentum* (lvs)	Melchers et al. (1978)	+	−
N. tabacum (lvs)	*L. esculentum* (lvs)	Turpin (1986)	+	?
P. hybrida (lvs)	*L. peruvianum* (lvs)	Tabaeizadeh et al. (1985)	+	?

?, No information
[a] Protoplasts were isolated from leaves (lvs), calli (c) or from suspension cells (scc)
[b] RC, Calli regenerating hybrid shoots
[c] GS, Germinating seeds

between these plants and wild-type plants indicated maternal inheritance of the mutation.

5. Somatic hybridization in the genus *Lycopersicon*

5.1 Early protoplast fusion experiments involving tomato

In 1970 Power et al. described the fusion of isolated plant protoplasts. It was the first step towards somatic hybridization. As early as 1978 somatic hybrids were created between *Solanum tuberosum* and *L. esculentum* var 'cerasiform' by Melchers et al. Tomato-potato hybrids have not yet been produced by sexual crossing, and this success demonstrated that somatic hybridization could be of great value for plant breeding. Later, somatic hybridization between tomato and its wild relative, *L. peruvianum*, was reported (Kinsara et al. 1986). The author observed that the regeneration capacity of tomato protoplasts was not a prerequisite for obtaining somatic hybrids between tomato and a wild species of *Lycopersicon*. Several wild *Lycopersicon* species are easy to regenerate from protoplasts, and this concept of unilateral regenerative capacity should be applied to numerous combinations involving tomato. In 1985 Adams and Quiros described a double selection scheme based on regeneration ability and antibiotic resistance. Thus, by the mid-1980s techniques were available for obtaining somatic hybrid tomato plants that combine two complete genomes (hybrids) and/or plants with a new nucleus-organelle combination (cybrids).

5.2 Species involved in somatic hybridization experiments

Somatic hybridization experiments have been made both within *Lycopersicon* and between *Lycopersicon* species and some species of other *Solanaceae* (Table 3).

Studies on somatic combination between *Lycopersicon* species and related or remote species showed that it is quite possible to use protoplast fusions for creating hybrid plants between sexually incompatible species (Table 3). It is important to note that in the regeneration of somatic hybrid plants, the physiological state of the plant materials appears to be one of the more important factors influencing the isolation and the fusion of protoplasts, followed by shoot development on hybrid calli (Turpin 1986). Somatic hybrid plants generally combine two different complete genomes. Numerous plants have been categorized as being symmetric hybrids (Schiller et al. 1982; O'Connell and Hanson 1986, 1987; Handley et al. 1986; Kinsara et al. 1986; Han San et al. 1990; Sakomoto and Taguchi 1991; Bonnema and O'Connell 1992). Some combinations have been found to spontaneously produce asymmetric hybrids. In fusions between *L. peruvianum* (2n = 24) and *P. hybrida* (2n = 14), the elimination of a certain number of *Petunia* chromosomes has been observed (Tabaeizadeh et al. 1985). In somatic hybrids between tomato (2n = 24) and tobacco (2n = 48), the chromosome number varied between 46 and 55 (Turpin 1986). In *Brassicaceae*, a positive correlation between the frequency of hybrids with eliminated chromosomes and the genetic distance between the parents has been found (Sundberg and Glimelius 1991).

Studies on the development of heterokaryons in culture suggested that significant karyological changes occur shortly after fusion. Gleba et al. (1987) analyzed the spatial arrangement of parental genomes in interspecific, intergeneric and intertribal hybrids of somatic cells and observed that the parental genomes in regenerating protoplast fusion products always enter the first cell division almost simultaneously, while the chromosomes of each parent form their own separated cluster within the com-

Table 4. Comparison of fertility of somatic hybrids

Species combination			Somatic PV	Hybrid SF	Plant CF	F_1 hybrid
(1)	*L. peruvianum*	*L. pennellii*	3%	–	–	Fertile
(2)	*L. peruvianum*	*L. esculentum*	43–45%	+	+	Sterile
(3)	*L. pennellii*	*L. esculentum*	<15%	–	–	Fertile
(4)	*S. lycopersicoides*	*L. esculentum*	2–49%	?	?	Sterile

?, No information
PV, Pollen viability; SF, self-fertility; CF, cross-fertility
(1) Adams and Quiros (1985); (2) Kinsara et al. (1986); (3) O'Connell and Hanson (1987); (4) Handley et al. (1986)

mon metaphase plate. Of particular interest is the observation that the parental genomes remain spatially separated within the nuclei even in regenerated hybrid plants. This study is of great practical significance since a priori chromosomal recombinations may occur only between chromosomes that are in close proximity.

5.3 Selection of fusion products

In protoplast fusion experiments, a major problem is the selection of fusion products. The fusion of different types of protoplasts (isolated from leaves and cell suspensions) makes possible the visual selection of fusion products (Glimelius 1985; O'Connell and Hanson 1985). There are numerous problems with this method. In somatic hybridization between mesophyll protoplasts of tomato and suspension culture protoplasts of different species (*L. peruvianum*, *S. lycopersicoides* and *S. rickii*), hybrid plants have been regenerated with variable chromosome numbers; some plants had the expected tetraploid number of chromosomes (4n = 48), and others had more than the tetraploid number. In somatic hybridization experiments between tomato and *S. rickii*, a single somatic hybrid callus was identified, which was chimeric with respect to the ploidy of the plants regenerated from it (2n = 48 to 130). It was suggested that the high ploidy level of calli obtained in previous somatic hybridization experiments between tomato and *L. pennellii* (O'Connell and Hanson 1985) was the reason for their inability to regeneration. Later, O'Connell and Hanson (1986) used *L. pennellii* protoplasts isolated from a fresh callus culture to obtain hybrid plants. An analysis of *S. lycopersicoides* suspension cells has shown that most of the chromosomal changes observed in somatic hybrids between this species and tomato could be ascribed to those that had occurred in the suspension culture (Moore and Sink; 1988 a, b).

Other selection techniques have been used depending on material and species. Protoplast fusion using a double selection scheme based on the regeneration ability of *L. peruvianum* and resistance to the antibiotic G418 in an *L. pennellii* cell line has been used to obtain somatic hybrids (Adams and Quiros 1985). In somatic hybridization experiments between tomato and *S. nigrum*, a two-step selection system was used based on differences in nutritional requirements. In such cases, (*L. esculentum* × *L. pennellii*) (+) *S. lycopersicoides* and *L. esculentum* (+) *S. muricatum*, hybrid calli exhibited more vigorous growth than the other ones, and this hybrid vigor facilitated their identification. O'Connell and Hanson (1987) identified somatic hybrid calli on the basis of heterozygous isozyme banding patterns, and only presumed fusion products were subcultured to produce shoots. It is difficult to compare the different selection systems and their efficiency, but the use of selectable markers such as antibiotic resistance means that there is a permanent control over the fate of the traits to be transferred and perhaps a guarantee that they are maintained.

5.4 Meiosis in somatic hybrid plants

Somatic hybrids show a range in fertility, varying from self-fertile to highly sterile. In certain combinations, it has been possible to compare the fertility of F_1 hybrids and somatic hybrids (Table 4).

F_1 hybrids between *S. lycopersicoides* and *L. esculentum* have been found to be sterile, and somatic hybrids have set limited fruit (Handley et al. 1986). The high sterility of somatic hybrids between *L. pennellii* an *L. peruvianum* or *L. esculentum* precludes their utilization for further genetic analysis (Adams and Quiros 1985; O'Connell and Hanson 1987). The tetraploid somatic hybrids between *L. pennellii* and *L. peruvianum* were considered to be subvital, and it was not possible to assess their fertility due to their inability to flower. The hexaploid somatic hybrids displayed very abnormal meiosis, resulting in lagging chromosomes, and micronuclei in telophase II. Diploid sexual hybrids are fully fertile, and normal meiosis resulting in regular chromosome pairing was observed (Quiros 1986). On the contrary, while F_1 hybrids between tomato and *L. peruvianum* are mostly sterile, somatic hybrids were self-fertile and plants could be backcrossed with *L. esculentum* as the maternal parent (Kinsara et al. 1986). In tetraploid somatic hybrids, by metaphase I most of the quadrivalents were split into two with a regular arrangement along the equatorial plane; therefore, anaphasic separation resulted in 24 chromosomes separating to each pole. In hexaploid

Table 5. Somatic hybrid pollen viability

Species	Combination	PV	Authors
S. muricatum	*L. esculentum*	1%	Sakomoto and Taguchi (1991)
S. tuberosum	*L. esculentum*	?	Shepard et al. (1983)
P. hybrida	*L. peruvianum*	0%	Tabaeizadeh et al. (1985)
N. tabacum	*L. esculentum*	?	Hassanpour-Estahbanati and Demarly (1986)

somatic hybrids, by late metaphase the multivalents had dissociated, so anaphasic cells with a 36-36 separation could be obtained. Results presented by Wijbrandi (1989) confirm the good fertility of tetraploid somatic hybrids between tomato and *L. peruvianum* and provide some indications for tetrasomic inheritance of several traits in the hybrids. The production of fertile somatic hybrids constitutes a way to transfer certain "wild" traits into tomato. The arrangement of chromosomes in nuclei at fertilization effectively allows chromosomal recombinations (Ashley and Pocock 1981), and the use of genetic markers would permit the selection of hybrid progenies of interest.

In the case of intergeneric and intertribal somatic hybrids, the pollen viability is very low (Table 5). It has been suggested that cytoplasmic male-sterile plants have been regenerated (Hassanpour-Estahbanati et al. 1986; Turpin 1986), but no more information is yet available.

Fertility and chromosome stability in *Brassica napus* resynthesised by protoplast fusion have been investigated (Sundberg et al. 1987). It was concluded that seed set was very low for the hybrids with a chromosome number deviating from the sum of the two parents. In 1983 Shepard et al. produced somatic hybrid plants from fusions between chlorophyll-deficient protoplasts of a variegating protoclone of potato (2n = 48) and two different cultivars of tomato (2n = 24). These plants were cytologically examined at meiosis and mitosis. Results indicate a degree of mitotic instability and chromosome segregation during vegetative propagation, but not wholesale chromosome elimination. In contrast, there was clear evidence of chromosome elimination at meiosis. If all intergeneric and intertribal hybrids involving tomato are affected in the same manner, they will be of little use for transferring interesting traits from sexually incompatible species into tomato.

5.5 The transfer of desirable traits

In the genus *Lycopersicon*, the maintenance of double diploid or amphidiploid chromosome sets was characteristic for several intra- and interspecific somatic cell hybrids obtained with more or less related parents (see section 2). Since the donor (often a wild species) contains not only the trait of interest, but also many unfavorable genes, the transfer of only a small part of the donor genome to the recipient species would be better. In some fusion combinations, a degree of spontaneous genomic instability was detected; the practical usefulness of this plant material is reduced by its failure to produce sexual progenies (see section 2). Enforced and directed chromosome elimination would be extremely useful for hybrid plant production for breeding purposes. Irradiation has been successfully used to achieve nuclear gene transfer via asymmetric hybridization (Gupta et al. 1984; Bates et al. 1987).

5.5.1 Effect of radiation dose on the production of tomato somatic hybrids

In a first attempt to create asymmetric tomato hybrids, fusions were performed between tomato protoplasts and protoplasts of *L. pennellii* that had been exposed to 3 or 6 krad of gamma radiation (O'Connell and Hanson 1987). Plants regenerated following the fusion showed the same kind of heterozygous isozyme patterns as symmetric hybrids. It was suggested that radiation doses were not sufficient to inactivate this wild nuclear genome. The use of mapped isozymes and RFLP markers for the molecular characterization of somatic hybrids has finally shown that two of them are asymmetric (Melzer and O'Connell 1990). The two asymmetric hybrids have lost both copies of chromosome 12 of tomato. In addition, one of them has lost both copies of tomato chromosome 1. The elimination of tomato chromosomes from the two somatic hybrids may reflect asynchrony of parental nuclei. Loci on chromosome 2 from both somatic hybrids presented altered stoichiometry, with *L. pennellii* alleles being 4 times more abundant than expected. This apparent amplification of *L. pennellii* chromosome 2 is similar to the variation in ribosomal RNA gene copy number observed in somatic hybrids involving tomato and *S. lycopersicoides* (Moore and Sink 1988b). This phenomenon may be due to culture in vitro (Negrutiu et al. 1989).

More recently, asymmetric somatic hybrids between tomato and *L. pennellii* have been effectively obtained (Melzer and O'Connell 1992). Tomato protoplasts were treated with iodoacetamide to inhibit cell division, and protoplasts from the wild species were irradiated with either 5, 10, 15, 25, 50 or 100 krad of gamma radiation. Asymmetric hybrids between tomato and *L. pennellii* possessed about 48 chromosomes (fusions involving 15- and 25-krad-irradiated protoplasts) or 24 chromosomes (fusions involving 50- and 100-krad-irradiated protoplasts) (Table 6).

Asymmetric somatic hybrids of tomato and *L. peruvianum* have been obtained by the fusion of leaf protoplasts from both species after irradiation of protoplasts

Table 6. Production of asymmetric tomato hybrids

Fusion partners[a]		Resultant plants
L. esc. (io.tr.) (+)	*L. pennellii* (1)	
	5 krad	Full somatic hybrids
	10 krad	Full somatic hybrids
	15 krad	Asymmetric somatic hybrids (2n≈48)
	25 krad	Asymmetric somatic hybrids (2n≈48)
	50 krad	Asymmetric somatic hybrids (2n≈24)
	100 krad	Asymmetric somatic hybrids (2n≈24)
L. esc. (n.reg.) (+)	*L. peruvianum* (2)	
	5 krad	Triploid plants (2n≈30)
	30 krad	Pentaploid plants
	100 krad	Pentaploid plants

(1) Melzer and O'Connell (1992); (2) Wijbrandi et al. (1990a, b)
[a] Tomato protoplasts do not regenerate whole plants (n.reg.) or are treated with iodoacetamide to inhibit the cell division (io.tr.)

or leaf tissue of *L. peruvianum* with 5, 30 or 100 krad of gamma rays (Wijbrandi et al. 1990a, b). The asymmetric hybrids were selected on the basis of the superior regeneration ability of *L. peruvianum*. The ploidy level, morphology and regeneration rate of the asymmetric hybrids were analyzed in relation to the radiation dose applied to *L. peruvianum* (Table 6). Curiously, chromosome numbers of the asymmetric somatic hybrids between tomato and *L. peruvianum* varied from 29 to 85. The retention of three different types of genes or alleles was analyzed (Wijbrandi 1989): the dominant gene coding for kanamycin resistance introduced in *L. peruvianum* plants by transformation, genes coding for acid-phosphatase and glutamate oxaloacetate transaminase and 18 single genes responsible for morphological markers, for which the tomato genotype used was homozygous recessive. The results confirmed the limited elimination of the donor genome and are in contrast with the situation in asymmetric hybrids between tomato and *L. pennellii*, which retain only a few donor chromosomes (Melzer and O'Connell 1992).

It is important to note that a relationship between radiation dose and the extent of hybridity in the regenerants has been observed. Generally, the treatment determines the direction of elimination, but not the extent of elimination of the irradiated genomes (Negrutiu et al. 1989). In *Lycopersicon*, there is a dose effect for the frequency of asymmetric hybrids in the population of regenerants, and there is a trend for a decrease in the extent of hybridity per individual as a function of increasing radiation dose.

The double inactivation process used by Melzer and O'Connell (1992) seems to be more efficient for producing asymmetric hybrids. Several of their asymmetric hybrids are hybrid at only limited regions of the genome and are self-fertile. These individuals are candidates for further screening in tomato breeding programs. The production of asymmetric somatic hybrids between tomato and *L. peruvianum* was based on the regeneration capacity derived from the irradiated species (Wijbrandi et al. 1988). The polygenic nature of the selectable donor traits is probably the cause of the limited elimination of the *L. peruvianum* genome. These asymmetric hybrids are sterile and inaccessible to backcrossing.

5.5.2 Chromosomal analysis of asymmetric tomato hybrids

In the two kinds of tomato asymmetric hybrids, a few alleles of the recipient tomato genome, and in some cases even complete chromosomes, were absent. It is possible that exchange events with *L. peruvianum* chromosomes or chromosome fragments have taken place after fusion, followed by elimination of translocated parts. Another explanation for the loss of tomato chromosomes and fragments might be rearrangements that occur during the tissue culture phase (Pijnacker et al. 1986; Lee and Phillips 1988).

The asymmetry of hybrids between tomato and *L. pennellii* has been studied at 20–24 loci using isozymes and restriction fragment length polymorphism (RFLP). Individuals were considered to be asymmetric if they scored as hybrid at 1 locus and as tomato at another locus. Although the presence of both parental alleles is indicated by a hybrid score in the analysis, the physical location of the *L. pennellii* allele in the genome is not known. It could be carried on a tomato chromosome or on a *L. pennellii* mini-chromosome (Melzer and O'Connell 1992). In some cases it seems that the hybrid contains at least one intact copy of a *L. pennellii* chromosome in addition to the tomato homologs. In other cases, it is possible that a mini-chromosome derived from a *L. pennellii* chromosome is present in the hybrid; deleted or minichromosomes have been observed in combinations such as *N. plumbaginifolia* (+) *Atropa belladonna* or *N. sylvestris* (Gleba et al. 1988; Famelaer et al. 1989). And finally, there are cases where the *L. pennellii* allele probably recombines with a tomato chromosome. The genome composition of asymmetric somatic hybrids between tomato and *L. peruvianum* has been characterized by Southern blot analysis using 29 RFLP markers, representing the 12 chromosomes of *L. peruvianum*. All RFLP markers were recovered in at least 9 of the 15 asymmetric hybrids surveyed. The results provide substantial evidence that chromosome fragments generated by irradiation were involved in inducing rearrangements such as translocations (Wijbrandi et al. 1990b).

Protoplast fusion experiments involving a cytoplasmic albino tomato genotype and a potato genotype car-

Table 7. Experiments in tomato cybrid creation

Fusion partners		Authors	Nucleus	T.cp.	T.mt.
Tomato	*S. lycopersicoides*	Levi et al. (1988)	S.S.H.	97%	0%
Tomato	*L. peruvianum*	Han San et al. (1990)	S.S.H.	50%	10%
Tomato	*L. peruvianum* (irr.)	Derks et al. (1991)	A.S.H.	50%	50%
Tomato (c-d)	*L. peruvianum* (irr.)	Ratushnyak et al. (1991)	A.S.H.	0%	100%
Tomato	*L. pennellii* (irr.)	O'Connell and Hanson (1985)	?	–	0%
Tomato (io.tr.)	*L. pennellii* (irr.)	Bonnema et al. (1992)	Tomato	100%	0%
Tomato (c-d)	*S. tuberosum* (irr.)	Wolters et al. (1991)	A.S.H.	0%	?

Tomato protoplasts treated with iodoacetamide (io.tr.) or isolated from chlorophyll-deficient plants (c-d) were fused with irradiated "wild" protoplasts (irr.). Regenerated plants are symmetric somatic hybrids (S.S.H.), asymmetric somatic hybrids (A.S.H.), or have only the tomato chromosomes. The percentage of plants possessing tomato chloroplasts (T.cp.) and plants possessing tomato mitochondria (T.mt) are indicated in each experiment

rying the β-glucuronidase (GUS) gene of *Escherichia coli* have been carried out (Wolters et al. 1991). The potato protoplasts were isolated from plants irradiated with 5 or 50 krads of gamma rays. The isolation of hybrid calli was based on a cytoplasmically controlled trait of the donor species and not on the regeneration ability of the irradiated species. A limited degree of potato DNA elimination was observed in the asymmetric hybrids obtained.

5.6 Selection and analysis of cytoplasmic hybrids (cybrids)

Using crosses between a normal green plant and a plant expressing a chloroplast-encoded chlorophyll deficiency, Smith (1989) was able to determine that in tomato, plastids are inherited in a strictly uniparental-maternal fashion.

Somatic hybridization offers the possibility of manipulating chloroplastic and mitochondrial genomes (reviewed by Pelletier et al. 1988; Medgyesy 1989). Several combinations of cytoplasmic formations are theoretically possible in cybrids resulting from protoplast fusions between two parental lines (Kumar and Cocking 1987). Results obtained in cybridization within the genus *Lycopersicon* demonstrate that there are probably particular rules concerning the production of tomato cybrids (Table 7).

Results presented in Table 7 indicate the independent transmission of chloroplasts and mitochondria in somatic hybrids. The majority of hybrid plants have chloroplasts and mitochondria from opposite fusion parents, indicating that both organelles sorted out independently.

5.6.1 Chloroplastic genotype of hybrid plants

Chloroplast segregation generally occurs randomly in symmetric hybrid populations (O'Connell and Hanson 1986; Han San et al. 1990; Derks et al. 1991). Three types of plastidic composition have been detected in hybrid callus obtained by protoplast fusion between tomato and *L. pennellii*: either only tomato, only *L. pennellii* or a mixture of chloroplast DNA (cpDNA) (O'Connell and Hanson 1985). It seems that the predominance of one parental chloroplastic genome is already established in calli.

In contrast, biased plastid transmissions have been described in somatic hybrids between tomato and *S. lycopersicoides* (Levi et al. 1988) and between tomato and *S. nigrum* (Guri et al. 1988). Biased transmissions may result from an unequal initial input of chloroplasts, different rates of chloroplast replication or nucleus-chloroplast incompatibility. Tomato protoplasts are generally isolated from leaves, and it has been determined that mesophyll cells have more chloroplasts than those of any other tissue (Steele-Scott et al. 1984). For this reason, tomato chloroplasts could predominate in the heterocaryons obtained from the fusion of tomato mesophyll protoplasts and *S. lycopersicoides* suspension cell protoplasts. In *Oenothera*, differences have been observed in competition between the five plastome types when they are brought together by sexual matings (Schötz 1968). A similar phenomenon within the *Lycopersicon* genus could result in a biased transmission of chloroplasts in somatic hybrid plants. Genetic evidence for the incompatibility of plastomes with certain nuclear genomes has been obtained for *Oenothera*. In tomato (+) *S. nigrum* somatic hybrids, the biased sorting out of plastids could be due to a plastome-genome incompatibility since *L. esculentum* and *S. nigrum* are relatively remotely related.

5.6.2 Mitochondrial genotype of hybrid plants

In several cases there is evidence of a rapid elimination of tomato mitochondria in somatic hybrid plants (Shepard et al. 1983; Levi et al. 1988; Han San et al. 1990; Bonnema et al. 1991). Hybrid calli obtained from fusion experiments between tomato and *L. pennellii* already show the elimination of the tomato mitochondrial genome (O'Connell and Hanson 1985). It is possible that tomato-specific fragments are not detected (Levi et al. 1988). The dominance of the wild species mitochondrial DNA (mtDNA) could be caused by a higher replication rate of their mtDNA compared to tomato mtDNA. The number

of mitochondria per cell and the mtDNA ploidy may also influence mtDNA transmission (Levi et al. 1988). It seems that the parental mitochondria are not maintained as a mixture. In contrast, rearrangement events in mtDNA are facilitated by protoplast fusion (Belliard et al. 1979). In *Lycopersicon* and more or less related genera, there is evidence for mtDNA rearrangements in numerous somatic combinations (O'Connell and Hanson 1985; O'Connell and Hanson 1986; Guri et al. 1988; Levi et al. 1988; Derks et al. 1991; Melchers et al. 1992). Though evidence for mtDNA rearrangements has been reported in all cases, the presence of novel restriction fragments has been described only in three tomato – *S. nigrum* hybrids and one tomato – *S. lycopersicoides* hybrid. Further analysis of mtDNA is needed to determine whether these novel bands occurred following intragenomic or intergenomic recombinations. In 1990 intergenomic recombination was demonstrated in somatic hybrids between tomato and *L. peruvianum* (Han San et al. 1990). A limited number of recombinant mitochondrial hybridization patterns has been found in each of these somatic combinations, and the presence of hot spots or specific sequences for recombinations and/or rearrangements was suggested. This has also been described for *Brassica* (Vedel et al. 1986).

The analysis of organelle genomes in somatic hybrid indicates that novel organelle-nuclear combinations can effectively be created by somatic hybridization in *Lycopersicon.*

5.6.3 Construction of tomato cybrids

In the Solanaceous species, some preliminary information on genome/plasmone incompatibility was revealed by intergeneric somatic hybridization experiments, and the influences of the tomato nucleus and cytoplasm have been separately investigated by using subprotoplasts (Binding 1976). Within *Lycopersicon*, it seems that the production of true cybrids, fusion products possessing the nuclear genome of only one of the parents and a new organellar combination (Kumar and Cocking 1987), is difficult to obtain. Two groups have claimed to have regenerated cybrids following the fusion of tomato protoplasts and protoplasts from a wild *Lycopersicon* species (Bonnema et al. 1991; Ratushnyak et al. 1991). Bonnema et al. constructed cybrids by fusing iodoacetamide-treated leaf protoplasts of tomato and irradiated *L. pennellii* protoplasts. Cybrids were recovered at a frequency of 19%. All of the cybrids had a diploid chromosome number of 24, tomato chloroplasts and varying amounts of *L. pennellii* mitochondrial DNA. The organization of the mitochondrial genome in the somatic hybrids and cybrids has been compared in order to assess the role of the nuclear genotype in the inheritance of the mitochondrial genome (Wachocki et al. 1991). There was no difference in the average frequency of rearranged mitochondrial sequences in somatic hybrids versus cybrids, but the frequency of tomato-specific mtDNA sequences is higher in cybrids. One explanation is the influence of the nuclear genome.

Ratushnyak et al. (1991) have obtained cybrids by fusing protoplasts isolated from a chlorophyll-deficient tomato genotype and irradiated *L. peruvianum* protoplasts. The nuclear and cytoplasmic constitution of seven different regenerants has been studied. Only one callus has regenerated plants possessing tomato chromosomes and *L. peruvianum* plastids. The selection pressure applied during culture based on chlorophyll deficiency seems to be inefficient for selecting persistence of the "wild" cytoplasm in the fusion products. Tomato-potato hybrids have been obtained by the fusion of protoplasts of a cytoplasmic albino tomato genotype with potato protoplasts (Wolters et al. 1991). In this case, it is remarkable that a certain number of potato nuclear traits are required for the production of somatic hybrids containing potato plastids. Derks et al. (in preparation) could not obtain true cybrids when albino tomato protoplasts were fused with *S. commersonii* or *S. etuberosum* protoplasts. Similarly, Jain et al. (1988) could not isolate cybrids containing the tomato nucleus with atrazine-resistant chloroplasts derived from *S. nigrum.* In the *Brassicaceae*, Sundberg et al. (1991) observed that the somatic hybrids produced between distantly related species tend to favor the chloroplasts contributed by the species predominating in the hybrid nucleus (Sundberg and Glimelius 1991). The organellar genotypees of tomato (+) *L. pennellii* asymmetric somatic hybrids have been analyzed, and the results suggest that the nuclear background indeed exerts an influence on chloroplast segregation (Bonnema et al. 1992). In contrast to these results, it has been observed that chloroplasts sorted out randomly in asymmetric fusion products between tomato and *L. peruvianum* (Derks et al. 1991). In this case, there is also evidence for limited donor genome elimination.

Although the pollen fertility of the plants was reduced, all cybrids obtained by protoplast fusion between tomato and *L. pennellii* set fruit containing viable seeds after self-pollination (Bonnema et al. 1991). The presence of part of the *L. pennellii* mitochondrial genome in a tomato nuclear background did not induce male sterility. A first attempt to create cytoplasmic male sterility (CMS) in tomato by sexual crosses also failed (Mutschler 1989). Fruits from the cybrids recovered in Ratushnyak's experiments (1991) were parthenocarpic, with abortive embryos. Pollen viability was not discussed. Finally, Melchers et al. (1992) described one-step generation of CMS by fusion of mitochondrial-inactivated tomato protoplasts with nuclear-inactivated *Solanum* protoplasts. Analysis of mitochondrial DNA revealed that the mitochondrial genome of the CMS hybrids does not con-

tain all elements of the mtDNA of either, but includes sequences of a recombinational nature not present in either parent. The fusion of mitochondrial-inactivated tomato protoplasts with nuclear-inactivated protoplasts from *S. Lycopersicoides* or *N. tabaccum* did not produce male-sterile tomatoes (Melchers et al. 1992). These results suggest that the phylogenetical distance between fusion partners are of great importance in order to obtain CMS in tomato.

The majority of chloroplast proteins are nuclear in origin (Sugiura 1992), and it is possible that chloroplasts require nuclear-encoded chloroplast proteins from the same or a very closely related species in order to be functional. The interaction between the nuclear genome of a given species with plastomes of an alien species has been investigated in *Solanum* (Perl et al. 1991). There is a positive correlation between phylogenetic proximity and the successful transfer of organelles from a donor to a recipient species.

6 Conclusion

Symmetric somatic hybrids are relatively easy to obtain in *Lycopersicon*. In some cases, plants obtained from protoplast fusion have a different behavior in crosses than the corresponding sexual hybrids that are sterile (Kinsara et al. 1986), and thus could be used to introgress "wild" genes into tomato. However, symmetric hybrids are always polyploid plants and their use as tools in breeding programs is problematic. On the other hand, the transfer of interesting agronomic characteristics from wild *Lycopersicon* species to tomato by sexual crosses has been largely investigated, and numerous resistance genes to pests and diseases have been incorporated into commercial cultivars (H. Laterrot, personal communication). Finally, the recovery of symmetric hybrids does not seem to be of great interest in comparison with sexual hybridization.

The production of asymmetric hybrids between tomato and certain wild *Lycopersicon* species is of interest (Melzer and O'Connell 1992). The plants are fertile, and it seems probable that the presence of interesting agronomic characteristics can be analyzed; *L. esculentum* is a species in which numerous genetic markers are known and mapped (Mutschler and Tanksley 1990). By exploiting random recombination events between the fragmented genome of the wild species with the intact genome of the cultivar, it seems to be possible to introduce fragments of "wild" chromosomes into tomato. In the field of asymmetric hybridization, the production of asymmetric hybrids between tomato and incompatible wild *Solanum* species is of particular interest due to their specific characteristics such as arthropod resistance.

It is important to note that unilateral incompatibility between some *Lycopersicon* species seems to persist, in that tomato cybrids are produced with low efficiency. The production of new nuclear-cytoplasmic combinations is difficult to obtain. But Melchers et al. (1992) have concluded that tomato cybrids produced by the fusion of mitochondrial-inactivated tomato protoplasts with nuclear-inactivated *Solanum* protoplasts present unique traits such as the cytoplasmic male sterility.

Therefore, somatic hybridization is still emerging as a complementary technique in tomato breeding (Zelcer et al. 1990). Further studies should determine the limits of its potential applications.

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