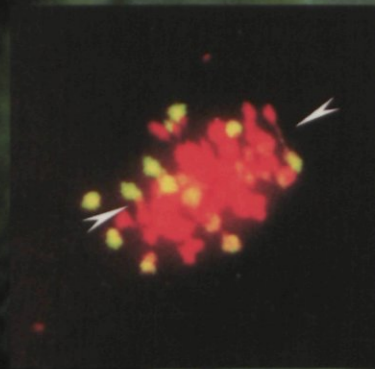


Introgression of tomato chromosomes into the potato genome: an analysis through molecular marker and *in situ* hybridisation techniques



Francesc Garriga Calderé

Introgression of tomato chromosomes into the potato genome: an analysis through molecular marker and *in situ* hybridisation techniques

Introgressie van tomatenchromosomen in het aardappelgenoom: een analyse met behulp van moleculaire merker en *in situ* hybridisatie technieken

Promoter: dr. ir. E. Jacobsen
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in het bijzonder in de genetische variatie en reproductie

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Propositions

1. Chromosome transfer across intergeneric boundaries such as *Solanum* and *Lycopersicon* can be accomplished (this thesis).
2. There is a potential to create a complete series of alien tomato chromosome additions into the potato genome (this thesis).
3. The frequencies of female transmission of individual tomato chromosomes to backcross progenies differ, but also depend on the genotypes of the parents (this thesis).
4. Non-sister chromatids can participate in the origin of a disomic addition (this thesis).
5. Homoeologous chromosome pairing between the potato and tomato genomes in the fusion hybrid is not abundant but introgression is possible (this thesis).
6. *In situ* hybridisation techniques have altered the face of plant cytogenetics by adding colour and accuracy.
7. The threat of not having welfare benefits has been a good stimulus for foreign students at WAU to wind up their Ph.D. thesis quicker than the Dutch students.
8. Traditional universities appear to be becoming out of place in the modern age of market forces and competition.
9. In spite of the realisation that "A full description of our genome will not be sufficient to understand its functional organisation, neither for individual units nor at a more integrated level" (Science editorial vol. 278, 1997), Human Genome Project is frantically persuaded.
10. Instead of being alarmed about the cloning of the sheep "Dolly", we should make a realistic assessment of the prospects that await us.
11. Whether genetically modified crops should be accepted or rejected is debatable, but the consumers have a right to know about the stuff they eat.
12. Industrialised countries should assume a greater responsibility to save the mankind from the disastrous greenhouse effects.
13. Nothing is pushing up more than the real necessity (Catalan proverb).

These propositions are a part of the thesis, "**Introgression of tomato chromosomes into the potato genome: an analysis through molecular marker and *in situ* hybridisation techniques**" by Francesc Garriga Calderé, Wageningen, Wednesday 18 March 1998.

Francesc Garriga Calderé

**Introgression of tomato chromosomes into the
potato genome: an analysis through molecular
marker and *in situ* hybridisation techniques**

Proefschrift

**ter verkrijging van de graad van doctor
op gezag van de rector magnificus
van de Landbouwniversiteit Wageningen,
dr. C.M. Karssen,
in het openbaar te verdedigen
op woensdag 18 maart 1998
des namiddags te vier uur in de Aula**

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*To my parents for their love, care,
support and understanding*

*To Dr. M.S. Ramanna my co-promoter from
whom I learnt what science is and what is
not, with my full heart and the highest respect*

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Last but not least, I thank Valerie Hecht and Alejandro Pérez García for their help in translating the summaries.

Abstract

This thesis describes the behaviour of tomato chromosomes in a potato background. It has been elucidated through RFLP, GISH and FISH analyses that despite their preferential elimination in somatic fusion hybrids and their abnormal meiotic behaviour all of them (12 different) can be efficiently transmitted through gametes to the subsequent generations in backcrosses to tetraploid potato. Not all of them proved to be maternally transmitted with the same frequency to the progeny. In spite of this, it has been possible to select potato genotypes with monosomic alien tomato additions for several of them. The combination of the techniques mentioned above proved to be very powerful in identifying and characterising a deletion. The extent of homoeologous pairing and recombination between intact potato and tomato chromosomes proved to be very low. In spite of this, it is possible to introgress chromosomal DNA of tomato into the potato genome.

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1

General Introduction

Transfer of chromosomes and genes from distantly related species and genera has been an integral part of plant breeding. In the past, such transfers could be achieved only when the distantly related taxa were amenable for sexual hybridisation. When this difficult step was achieved, the hybrids were repeatedly backcrossed to the desirable genotype, or cultivars in most cases, in order to retain useful traits and eliminate the undesirable ones. This process obviously required enormous amounts of time and effort and the success was not always predictable.

Improvements in the techniques of hybridisation such as embryo rescue, protoplast fusion, or the so-called symmetric and asymmetric somatic hybridisation, opened the possibilities to hybridise more distantly related taxa with greater efficiency. These techniques, besides overcoming the limitations of sexual hybridisation, also provided opportunities to produce backcross progenies in numerous instances. These developments were indeed a great step forward for germplasm enhancement in several crops.

Although the difficulties of hybridisation and backcrossing can be overcome to a great extent, the problem of retaining the desirable genotypes in the backcross progenies and preserving useful chromosomes and genes in the subsequent process of selection persisted till recently. With the introduction of molecular markers approaches such as: restriction fragment length polymorphisms (RFLP), amplified fragment length polymorphisms (AFLP), randomly amplified polymorphic DNA (RAPD), variable number of tandem repeats (VNTR) and “microsatellites” or single sequence repeats (SSR), among others (reviewed by Karp et al. 1996), for genetic analysis and selection, the potential for greater efficiency of selection has been made possible. Besides these techniques, the introduction of the non-radioactive methods of *in situ* hybridisation in plants (Rayburn and Gill 1985), such as genomic *in situ* hybridisation (GISH) and fluorescent *in situ* hybridisation (FISH) (Heslop-Harrison et al. 1990) have opened entirely new perspectives for monitoring introgressed chromosomes and genes from distantly related taxa into the cultivated plants.

Table 1 Some recent examples of alien chromosome additions in crop plants in which molecular techniques have been involved

Taxa	Method of detection	Purpose	Reference
<i>Gossypium hirsutum</i>	GISH, FISH	Mapping	Ji et al. 1997
<i>Nicotiana plumbaginifolia</i> X <i>Lycopersicon esculentum</i>	Kanamycin resistance, cytological	Introgression	Vlahova et al. 1997
<i>Triticum aestivum</i> X <i>Agropyron elongation</i>	C-banding, GISH	Disease resistance	Cai et al. 1996
<i>Triticum durum</i> X <i>Agropyron elongation</i>	GISH	Disease resistance, protein analysis	Ceoloni et al. 1996
<i>Brassica napus</i> x <i>B. nigra</i>	RFLP, RAPD	Disease resistance	Chevre et al. 1996
<i>Beta vulgaris</i> X <i>B. procumbens</i>	Southern blotting	Introgression, disease resistance	Mesbah et al. 1996
<i>Beta vulgaris</i> X <i>B. patellaris</i>			
<i>Triticum aestivum</i> X <i>Secale cereale</i>	GISH	Mapping	Müller et al. 1996
<i>Avena sativa</i> X <i>Zea mays</i>	RFLP, GISH, FISH	Methodology	Riera-Lizarazu et al. 1996
<i>Triticum aestivum</i> X <i>Secale cereale</i>	RFLP, GISH	Methodology	Wang et al. 1996
<i>Allium cepa</i> X <i>A. fistulosum</i>	Isozyme, Karyotyping	Methodology	Shigyo et al. 1996
Wheat X <i>Haynaldia villosa</i>	C-banding, GISH	Disease resistance	Chen et al. 1995
<i>Solanum tuberosum</i> (+) X <i>Lycopersicon esculentum</i>	RFLP, GISH	Introgression	Jacobsen et al. 1995
<i>Avena sativa</i>	GISH	Phylogeny	Leggett and Markand 1995
<i>Oryza sativa</i> X <i>O. australiensis</i>	RFLP	Introgression	Multami et al. 1994
<i>Nicotiana plumbaginifolia</i> X <i>N. sylvestris</i>	RFLP	Synteny	Suen et al. 1994
Radish X kale	Cytological	Introgression	Kaneko et al. 1992
<i>Triticum aestivum</i> X <i>Leymus multicaulis</i>			
<i>T. aestivum</i> X <i>Thinopyrum bessarabicum</i>	GISH	Mapping	Schwarzacher et al. 1992
<i>Hordeum vulgare</i> X <i>Secale cereale</i>			
(<i>Allium cepa</i> X <i>A. fistulosum</i>) X <i>A. cepa</i>	Isozyme	Introgression	Creyder et al. 1991
<i>Festuca dymyria</i> X <i>Lolium multiflorum</i>	Isozyme, Cytological	Introgression	Morgan 1991
<i>Brassica oleracea</i> X <i>B. campestris</i>	RFLP, Isozyme	Methodology	McGrath and Quiros 1990

Addition of the alien chromosomes into cultivated plants not only serves the purpose of introgression, but it also provides valuable material for molecular cytogenetic analysis. Some of the examples are: (i) increasing the accuracy of genetic maps (Multaini et al. 1994; Gill et al. 1996), (ii) physical mapping of chromosomes (Schwarzacher et al. 1992), (iii) study of meiotic behaviour of individual alien chromosomes (Baley et al. 1993; Thomas et al. 1994; Kamstra et al. in preparation), (iv) elucidation of the molecular organisation of individual chromosomes (Fransz et al. 1996; Zhong et al. 1996), (v) creation of individual chromosome libraries (Ananiev et al. 1997), and (vi) establishment of syntenic relationships among the genomes of related genera and families (Ji et al. 1997). In view of these attractive features, there has been a considerable increase of interest in establishing monosomic alien addition lines in several crops. Some of the examples are listed in Table 1.

A striking feature of the species mentioned in Table 1 is that a majority of them are monocots (cereals) in which wide hybridisation is relatively easy. In these cases, sexual methods of hybridisation have been used. In contrast, in dicots both sexual and para-sexual methods (somatic hybridisation) have been used for the production of alien chromosome addition lines. Besides sexual and para-sexual methods, anther culture and preferential chromosome elimination have also been used successfully. For example, in wheat x rye (*Triticale*) hybrids, anther culture was used for the production of rye chromosome additions in a wheat background (Wang et al. 1996). Selective chromosome elimination of maize chromosomes in oat x maize hybrids has been successfully exploited for the production of monosomic addition lines of oat with maize chromosomes (Riera-Lizarazu et al. 1996). In addition, the so-called microprotoplast fusion has been used to produce monosomic alien chromosome additions in one step (Ramulu et al. 1996).

Among Solanaceae taxa, both sexual and somatic fusion methods of hybridisation have been used. The intergeneric sexual hybrids *Lycopersicon esculentum* x *Solanum lycopersicoides* were produced a long time ago (Rick 1951). The amphydiploids of this hybrid, however, could only be backcrossed recently (Rick et al. 1988) to one of the species of *Lycopersicon*, i.e. *L. pennellii*. The triploid genotype resulting from this cross, the so-called sesquidiploid, was further backcrossed to *Lycopersicon* species in order to establish a complete series of alien

chromosome addition lines in which *S. lycopersicoides* chromosomes were added into the tomato genome. One advantage of crossing the sesquidiploids to a diploid genotype was that it was possible to establish alien chromosome addition lines at the diploid level. These diploid genotypes with alien chromosome additions are advantageous for several reasons: (i) for cytological investigations, the diploid or near diploid genotypes are more favourable, and (ii) alien chromosomes can be substituted in the diploid genotypes more easily. When the alien chromosomes, or their parts, are in a homozygous condition in an alien background, the characters of the alien parent can be expressed. For example, several traits of *S. lycopersicoides* have been expressed in a tomato background (Chetelat et al. 1989, 1997).

Besides sexual methods, both symmetric and asymmetric protoplast fusion techniques have been used for the production of intergeneric hybrids in some Solanaceous taxa. Among others, potato (+) tomato (Melchers et al. 1978; Shepard et al. 1983; Jacobsen et al. 1992; Schoenmakers et al. 1992), tobacco (+) tomato (Hassanpour-Estahbanati et al. 1986; Turpin 1986), tomato (+) *Solanum muricatum* (Sakomoto and Taguchi 1991), tomato (+) *S. etuberosum* (Derks et al. 1992; Gavrilenko et al. 1992) are some of the examples reviewed by Wolters et al. (1994).

Despite the production of a number of these somatic fusion hybrids, there have been very few reports on attempts to backcross them to any of the parents. One exception, however, is the successful backcrossing of a potato (+) tomato somatic hybrid to potato (Jacobsen et al. 1994). This breakthrough opened the prospect for the establishment of monosomic alien additions of tomato chromosomes into potato genomes as well as introgression. Although the initial results were encouraging, the preferential elimination of alien tomato chromosomes from the somatic fusion hybrids to the BC₁ and BC₂ progenies generated a problem because only six different tomato chromosomes, among the twelve possible, were retained (Jacobsen et al. 1995). This was obviously a drawback for establishing a complete monosomic alien tomato addition series from the initial backcross material. Therefore, further efforts in order to create the potential for establishing a complete series of alien tomato chromosome additions into the potato genome were essential. Moreover, clear insights into the rate of transmission of alien chromosomes to the backcross progenies, the behaviour of the alien tomato chromosomes in the potato genome, the possibilities of homoeologous

pairing and crossing-over between the chromosomes of the two genomes and the structural integrity of the alien additions were required.

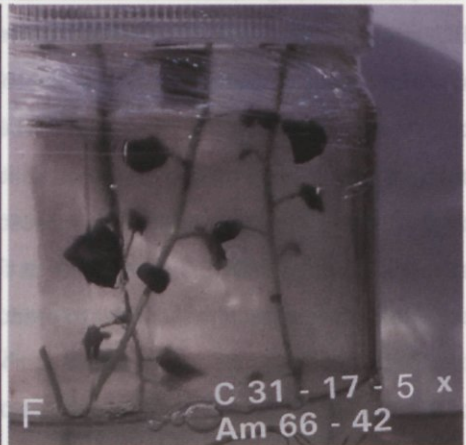
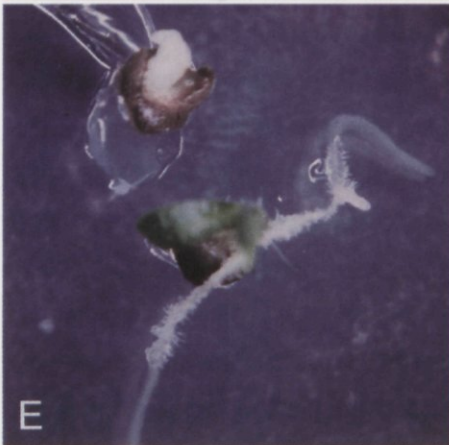
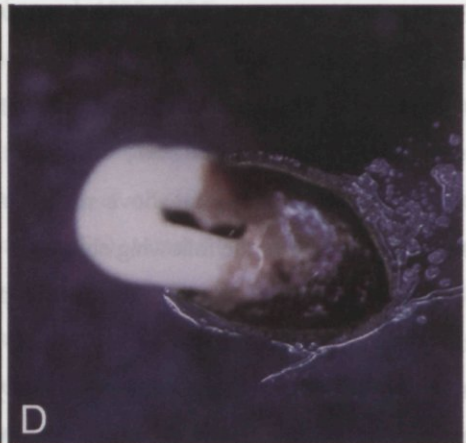
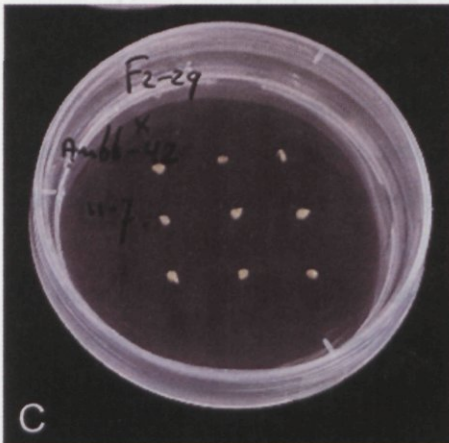
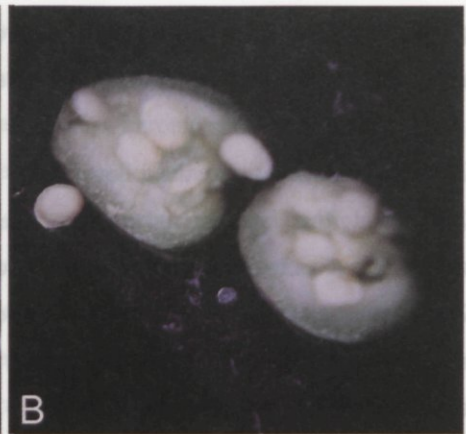
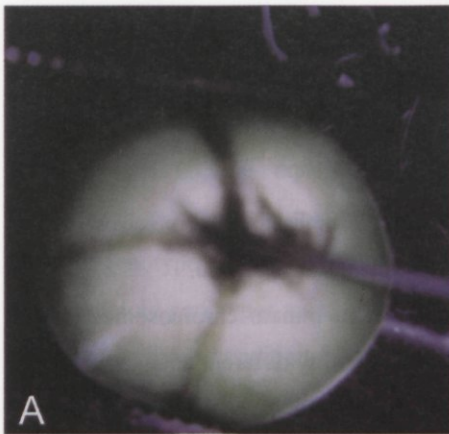
The aims of this thesis are the following:

1. To create the plant material with the potential for establishing a complete series of monosomic alien tomato chromosome additions in the potato genome.
2. To investigate the rate of transmission of the alien tomato chromosomes to the backcross progenies, their meiotic behaviour and to identify different alien monosomic tomato additions.
3. To elucidate the occurrence and behaviour of certain anomalous alien tomato chromosomes in the backcross progenies.
4. To assess the possibility of introgression through homoeologous pairing.

In order to fulfil the above stated aims, several investigations were conducted and are described in the following chapters:

In chapter 2, the results on the production of new backcross genotypes between the potato (*Solanum tuberosum*) (+) tomato (*Lycopersicon esculentum*) somatic fusion hybrids and different genotypes of tetraploid *S. tuberosum* through embryo rescue (Fig. 1) are described. The main aim of this investigation was to select BC₁ progenies that can offer the full potential for the establishment of a complete series of monosomic alien tomato chromosome additions into the potato genome. By characterising several BC₁ progenies through a combination of RFLP and GISH analyses for the alien tomato chromosome identification, genotypes with the potential to produce all the 12 possible alien monosomic tomato chromosome additions were selected. Three of these BC₁s were used as female parent and backcrossed to different tetraploid potato genotypes in order to produce BC₂ progenies.

In chapter 3, the results of the combined RFLP and GISH analyses on a total of 97 BC₂ progeny plants with the purpose of identifying potato genotypes with single alien tomato chromosome additions are presented. Besides identifying seven different monosomic tomato chromosome additions, viz., 1, 2, 4, 6, 8, 10 and 12, the rate of female transmission of alien chromosomes was determined and a statistical analysis was conducted.



In chapter 4, a detailed cytological analysis of an alien disomic addition, in which one of the homologues was much smaller (aberrant) than the normal counterpart, is presented. Through a detailed analysis, which included GISH and FISH, of chromosome morphology and pairing behaviour it was established that the aberrant chromosome had suffered an interstitial deletion on the long arm of the tomato chromosome 10. Based on an analysis of a BC₃ population through RFLP and GISH analyses, the rate of transmission of the normal and aberrant tomato homologue was estimated. In addition, an extensive study of microsporogenesis provided direct evidence for the mode of origin of alien tomato disomic additions in this material.

In chapter 5, the aspects of allosyndetic pairing and homoeologous recombination between the potato and the tomato chromosomes were investigated cytologically through GISH analysis. For this purpose, one of the somatic fusion hybrids that possessed two chromosomes with intergenomic translocations and two genotypes with alien tomato monosomic additions, viz., chromosome 1 and 8, were investigated. The results on the frequencies of allosyndetic chromosome associations at different stages of male meiosis are presented. Furthermore, the prospects for the introgression of tomato traits into potato are discussed.

In the final chapter 6, some of the general aspects on the establishment, use and significance of monosomic alien additions of tomato chromosomes into the potato genome are discussed.

Fig. 1 Chronology of the origin of BC1 2002. **A** A 24 days old berry from a cross between C31-17-5 X AM66.42. **B** Excised seeds. **C** Seeds placed on culture medium. **D** In vitro germinating seed after two months in culture. **E** Differentiated young plantlet. **F** Fully developed plant, 2002, multiplied in vitro

2

Identification of alien chromosomes through GISH and RFLP analysis and the potential for establishing potato lines with monosomic additions of tomato chromosomes

F. Garriga-Calderé, D.J. Huigen, F. Filotico, E. Jacobsen and M.S. Ramanna

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Abstract

To increase the potential for establishing a complete series of tomato chromosome addition-substitution lines in a potato background, six new BC₁ progeny were produced. All of them originated from crosses between three different hexaploid potato (+) tomato fusion hybrids. Three different somatic hybrids, viz., C31-17-5, C31-17-24, and C31-17-51, were used as female parents, and four different tetraploids, viz., Katahdin, Frieslander, 6704-1, and AM66.42 were used as male parents. A characterisation of the genomes of the three fusion hybrids and the six BC₁ progenies (6739, 2001, 2002, 2003, 2004, and 2005) through genomic *in situ* hybridization (GISH) and restriction fragment length polymorphism (RFLP) analysis indicated that there was preferential tomato chromosome elimination in the fusion hybrids. Similar analyses of the six BC₁ progeny indicated that a variable number of the alien tomato chromosomes (6-11) were present in individual plants. RFLP analysis using chromosome-specific DNA probes indicated that BC₁ progenies had retained all 12 tomato chromosomes, albeit in different individual plants. This means that the available BC₁ progenies have the potential for establishing of a complete series of tomato chromosome addition-substitution lines in a potato background.

Introduction

The first successful somatic hybridization between *Solanum* and *Lycopersicon* species (Melchers et al. 1978) generated considerable optimism for transferring genes across the intergeneric boundaries within the Solanaceae. In reality, it proved to be much more difficult than expected, because it was not possible to backcross the fusion hybrids to any of the parents. This hurdle appeared to be overcome when the potato (+) tomato fusion hybrid was successfully backcrossed to potato for the first time (Jacobsen et al. 1994). On careful characterisation of the alien tomato chromosomes in the fusion hybrids and backcross progenies (BC₁ and BC₂) through genomic *in situ* hybridization (GISH) and restriction fragment length polymorphism (RFLP) analyses it was found that only 6 of the haploid set of 12 tomato chromosomes were retained in the BC progenies (Jacobsen et al. 1995). The absence of some of the alien tomato chromosomes was already evident in the only BC₁ progeny that was available. This was, once again, a setback from the point of view of introgression, and for establishing a complete series of tomato chromosome addition lines in a potato background.

To pave the way for establishing potato plants with a complete series of monosomic tomato chromosome additions, obviously, more BC₁ progenies were required. In this context, we produced additional BC₁ progenies using different parental genotypes and these were characterised through GISH and RFLP analyses. Besides establishing the chromosome constitution of the BC₁ progenies, meiotic chromosome pairing behaviour was also investigated. These results, together with a discussion on the prospects for producing tomato chromosome addition lines, form the subject of this article.

Materials and methods

The plant material consisted of the hexaploid ($2n = 6x = 72$) potato (+) tomato fusion hybrids and the tetraploid ($2n = 2x = 48$) potato. The fusion hybrids belonged to the C31-17 series and the details of their origin have been described previously (see Jacobsen et al. 1994). Three genotypes, viz., C31-17-5, C31-17-24, and C31-17-51, were used as female parents in the backcrossing programme. Based on GISH pilot

experiments, it was established that all these hexaploid fusion hybrids possessed four genomes of potato and two of tomato (PPPPTT). Several tetraploid potato genotypes were tested as pollen parents, but only four of them were successful: the cultivars Katahdin and Frieslander; a breeding clone, AM66.42; and a nulliplex genotype with amylose-free starch (*amf*), 6704-1 (Jacobsen et al. 1989).

In all crosses, the fusion hybrids were invariably used as female parents, in view of their total male sterility (based on the criterion of staining pollen with lactophenol acid fuchsin), and the tetraploids as male parents. The techniques of crossing and in vitro culture of immature seeds were those of Jacobsen et al. (1993), with slight modifications. The selection of young developing seeds for in vitro culture was more stringent in the present investigation than in the previous study. Developing seeds that were relatively more plump were the only ones used for in vitro culture (see Chap. 1, Fig. 1, pag. 6). Secondly, instead of using agar, gelrite was used in the medium, and the medium was refreshed every 14 days.

GISH

For analysing chromosome constitution, the root tips were harvested in the morning, treated in 2 mM 8-hydroxyquinoline for 2-5 h at 18°C, and fixed in 96% ethanol-100% acetic acid (3:1). For meiotic studies, young flower buds containing pollen mother cells at the appropriate stage were harvested; to monitor the stage of development one anther was squashed in a drop of aceto-carmin and examined under a phase contrast microscope, the remaining anthers were fixed in ethanol-acetic acid (3:1).

The protocol followed for digesting of both types of tissues with a pectolytic mixture was identical to that of Jacobsen et al. (1995). Chromosome spreads on a grease-free slides were prepared according to Pijnacker and Ferwerda (1984).

The procedure of DNA denaturation, *in situ* hybridization and detection were those of Schwarzacher and Heslop-Harrison (1994). Tomato DNA was sonicated to a fragment size of 5-10 kb and directly labelled with fluorescein-11-dUTP (Amersham), following a standard random primed labelling protocol. The potato DNA used for blocking was autoclaved for 5 min, giving a fragment size of 200-500 bp. The hybridization mixture (40 µL/slide) consisted of 50% deionized formamide, 10% (w/v)

sodium dextran sulphate (Sigma), 2 x SSC (1 x SSC: 0.15 M NaCl plus 0.015 M Sodium Citrate), 0.25% (w/v) SDS, 2.5 ng/ μ L probe DNA, and 0.1 μ g/ μ L blocking DNA.

The hybridization mixture was directly applied to the slides containing the chromosome spreads, covered with a cover slip, and denatured at 80°C for 2 min. Hybridization was performed overnight at 37°C and the slides were then washed in 2 x SSC buffer for 15 min at room temperature, in 0.1 X SSC for 30 min at 42°C and in 2 x SSC for 15 min at room temperature. The chromosomes were counterstained with 2 μ g/mL DAPI (4'6-diamidino-2-phenylindole) and 5 μ g/mL propidium iodide and mounted in 10 μ L antifade. Selected chromosome spreads were photographed on 400 iso colour negative film with an Axiophot microscope equipped with UV light and the appropriate filter block.

RFLP analysis

For RFLP analysis, DNA from young shoot tips and leaves was extracted according to Bernatsky and Tanksley (1986). The procedures for restriction digestion and Southern hybridization were those of Kreike et al. (1990). For reliable chromosome identification, the tomato chromosome-specific probes were selected on the basis of the following criteria: (i) the probe showed clear polymorphisms identifying both tomato and potato chromosomes in the parents; (ii) both polymorphic bands were clearly present in the fusion hybrid; (iii) the polymorphic band was absent in the pollen parent(s) used in backcrosses; and (iv) the probe unequivocally identified the tomato chromosome in the BC₁ progeny (Fig. 1).

The 12 probes corresponding to the tomato and potato chromosomes in order from 1 to 12 are TG53, TG34, TG130, TG500, TG23, TG115, TG143, TG160, TG8, TG285, Ssp29, TG28. The TG probes were kindly provide by S.D. Tanksley, Cornell University, N.Y., U.S.A and the Ssp probe by C.M. Kreike, Wageningen NL.

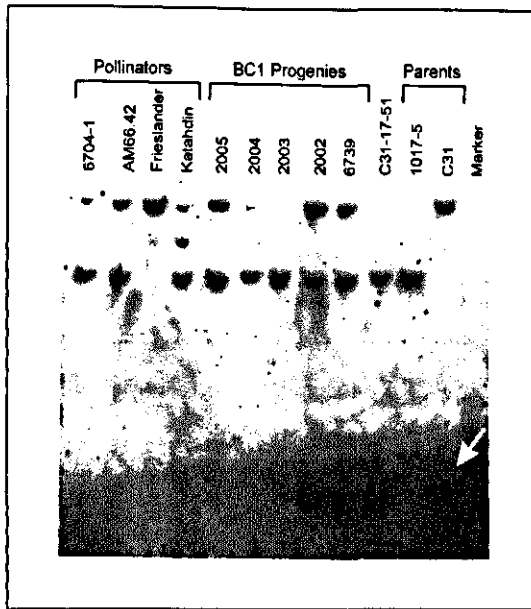


Fig. 1 A representative autoradiogram of a Southern blot after *Eco*RI digestion showing polymorphism for the probe TG28, which is specific to chromosome 12 of tomato. Note: The band is clearly visible in tomato, C31 (arrow), but absent in potato, 1017-5; both bands are present in the somatic fusion hybrid, C31-17-51. In two of the BC_1 progenies, 6739 and 2002, the marker is present whereas, in 2003, 2004, and 2005 it is absent. The four pollinators, Katahdin, Frieslander, AM66.42, and 6704-1, also lack this marker

Results

Production of BC_1 progenies

The hexaploid fusion hybrids were used as female parents for backcrossing in view of the previous success when the hexaploid genotype C31-17-1 gave rise to a single BC_1 progeny (Jacobsen et al. 1994). By using other genotypes from the same series of hybrids as female parents, a considerable number of seeds were obtained that could be used for in vitro culture. Of these, only a small percentage developed into viable BC_1 plants (Table 1). The results included in Table 1 represent only successful cases that were characterised and were directly relevant to this investigation. Generally, in other parental combinations of different genotypes, the rate of success was extremely low, and even when germination was obtained, development into adults plants did not occur (results not included).

Table 1 Berry and seed set in hexaploid fusion hybrids of diploid potato and tomato crossed with 4x potato clones as male parents in different growing seasons

Year	Genotype	No. of pollinators tested	No. of pollinations	No. of berries	No. of seeds	No. of plants obtained (%)
1993	C31-17-24	1	3	1	53	1 (1.9)
1994	C31-17-5	8	25	19	210	3 (1.4)
	C31-17-24	7	24	11	53	0 (0.0)
1995	C31-17-5	1	3	2	39	0 (0.0)
	C31-17-24	2	8	8	24	0 (0.0)
	C31-17-51	4	18	13	27	2 (7.4)
Total			88	55	406	6 (1.5)

Table 2 Parentage and pollen stainability of the BC₁ progenies analysed

BC ₁	Parentage of the BC ₁		Pollen stainability (%)
	Female	Male	
6739	C31-17-24	AM66.42	31.1
2001 ^a	C31-17-5	Katahdin	-
2002	C31-17-5	AM66.42	9.0
2003	C31-17-5	6704-1	13.1
2004 ^b	C31-17-51	Frieslander	-
2005	C31-17-51	AM66.42	28.1

^a Did not flower^b Flowers did not produce pollen

The six BC₁ plants originated from different parental combinations (Table 2) and all of them were grown successfully to the adult plant stage. Morphologically, the plants were more similar to potato, with some intermediate traits of tomato such as the shapes of leaves and flowers. In contrast to the uniform morphological features of fusion hybrids, the six BC₁ plants were quite different from each other with respect to growth characteristics, vigour, and flowering. One of the BC₁ plants did not flower, and among the five that did, one did not produce pollen. Among the four plants that produced pollen, the percentage of stainable pollen was variable (Table 2).

Genomic constitution of the fusion hybrids and BC₁ progenies

A combination of two different approaches was used in order to assess the genome constitution of the three fusion hybrids; C31-17-5, C31-17-24, and C31-17-51, and to identify the individual alien chromosomes in the six BC₁ progenies. These were (i) cytological, in which the genomes and chromosome numbers were distinguished

through GISH and (ii) genetic, in which the different chromosomes were identified through RFLP analysis using chromosome-specific DNA probes.

Fusion hybrids

Although in pilot experiments the three fusion hybrids were determined to be hexaploids (PPPPTT) through chromosome counting (Jacobsen et al. 1994), a more accurate confirmation of their genome composition was made through GISH from both mitotic and meiotic chromosome studies. Unexpectedly, the chromosome numbers among the fusion hybrids varied from $2n = 6x - 4 = 68$ to $2n = 6x = 72$ (Table 3). In all three fusion hybrids, the four genomes of potato were nearly intact, accounting for approximately 48 chromosomes as revealed by GISH. In two cases, C31-17-24 and C31-17-51 the number of tomato chromosomes deviated from the 24 that were expected to be present if the two genomes were intact (Figs. 1 and 2; Table 3). In view of this discrepancy, it was clear that the elimination of the chromosomes of tomato gave rise to aneuploidy in the fusion hybrids. A notable feature in one of the fusion hybrids, C31-17-51, was that among the 22 tomato chromosomes, 2 of them possessed segments of potato chromosomes of variable size (Fig. 2A). The presence of these two translocated chromosomes was confirmed through repeated observations in many cells. In Table 3 these two chromosomes are indicated as translocated chromosomes. Such translocated chromosomes were identified not only in the fusion hybrid, but also in the BC₁ progenies (Table 3; Fig. 2F, *arrow*). The one translocated chromosome in the BC₁ 2004 (Table 3) probably originated from its parent fusion hybrid, C31-17-51. However, the translocated chromosome in BC₁ 2001 originated from a different fusion hybrid, C31-17-5, that had normal complements, but 2001 possessed a clear exchange (Fig. 1F, *arrow*). It was, however, impossible to establish whether this was the result of homoeologous crossing-over or of spontaneous intergeneric chromosomal interchange.

BC₁ progenies

Normally, the BC₁ plants being the progeny of $6x - 4x$ crosses, they are expected to possess a pentaploid ($2n = 5x = 60$) chromosome number. However, in the present BC₁ progenies the chromosome numbers varied from $2n = 5x - 3 = 57$ to $2n = 5x = 60$

Table 3 The number of tomato chromosomes detected through GISH and identified through RFLP analysis in the somatic fusion hybrids and in the BC₁ progenies

Chromosome No.	Probe	Parents		Fusion hybrids ^a					BC ₁ progenies				
		Tomato	Potato	F-24	F-5	F-51	6739	2001	2002	2003	2004	2005	
		C31	87.1017/5										
1	TG53	+	-	+	+	+	+	+	+	+	+	+	+
2	TG34	+	-	+	+	+	+	+	+	+	+	+	+
3	TG130	+	-	+	+	+	+	+	-	+	+	+	+
4	TG500	+	-	+	+	+	+	+	+	+	-	+	+
5	TG23	+	-	+	+	+	+	+	+	+	-	+	+
6	TG115	+	-	+	+	+	+	+	+	+	+	+	+
7	TG143	+	-	+	+	+	+	+	+	+	+	+	+
8	TG160	+	-	+	+	+	+	+	+	+	+	+	+
9	TG8	+	-	+	+	+	+	+	+	+	+	+	+
10	TG285	+	-	+	+	+	+	+	+	+	+	+	+
11	Ssp29	+	-	+	+	+	+	+	+	+	+	+	-
12	TG28	+	-	+	+	+	+	+	+	+	+	+	-
	GISH ^b	24	0	23	24	20+2 ^t	11	9+1 ^t	12	12	11+1 ^t	10	
	RFLP ^c	12	-	12	12	12	11	8	11	10	6	9	
	2n ^d	24	24	71	72	68	59	57	60	60	58	58	

^a (F-24, C31-17-24; F-5, C31-17-5; F-51, C31-17-51)^b Number of tomato chromosomes detected by GISH; t indicates a translocated chromosome^c Number of tomato chromosomes detected through RFLP analysis^d Somatic chromosome number

(Table 3). In all cases, the four genomes of potato were intact, whereas the number of tomato chromosomes varied from $n = x - 3 = 9$ (Fig. 1F) to $n = x = 12$. The aneuploidy in the BC₁s was the result either of chromosome elimination, as in the case of fusion hybrids, or of incomplete transmission of the genome of tomato through the egg cells.

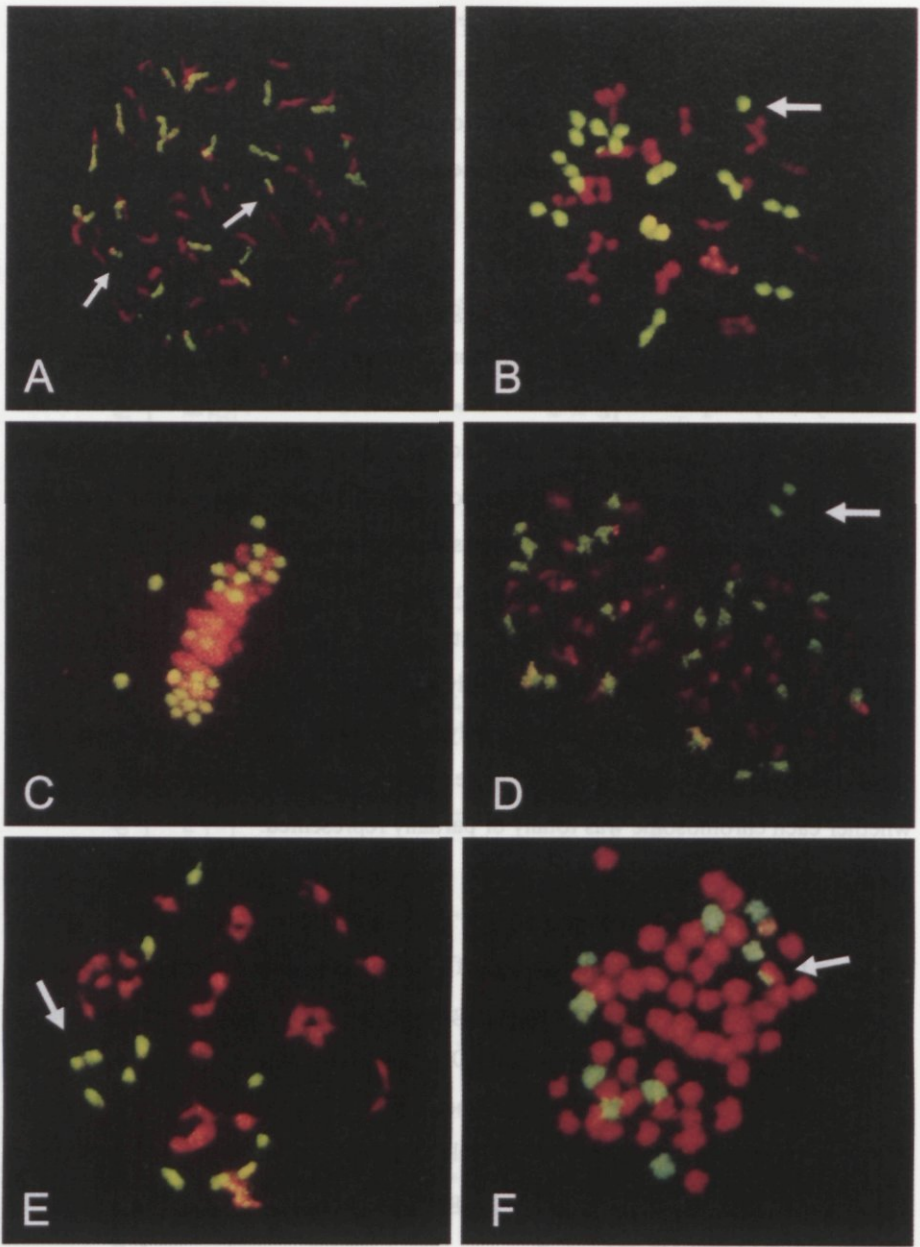
RFLP analysis in the fusion hybrids

Besides cytological identification of the genomes, RFLP analysis was helpful for identifying individual chromosomes in fusion hybrids and BC₁ progeny. In all the three fusion hybrids, despite aneuploidy in C31-17-51 and C31-17-24, the chromosome-specific-probes indicated that all 12 chromosomes of tomato were present (Table 3). In C31-17-51, for example, 2 of the 24 chromosomes of the tomato complement were absent and the other 2 possessed a translocation with potato, yet RFLP analysis identified the complete set of 12 tomato chromosomes. The inference was that in this genotype eight chromosomes were present in pairs (duplicate), with two present in single copies and two present in partially single copies, thus accounting for the complete set. As expected, RFLP analysis could not distinguish between the chromosomes that were in pairs or were single, nor was it possible to conclude whether each chromosome was totally or partially represented.

RFLP analysis in BC₁ progenies

Unlike in the fusion hybrids, the number of individual tomato chromosomes identified through RFLP analysis in the six BC₁ progenies was highly variable. For example, in genotype 2004, chromosome-specific DNA probes identified only 6 tomato chromosomes, whereas in 2 other genotypes, 2002 and 2003, there were 11 and 10 individual tomato chromosomes, respectively. A remarkable feature was that, based on RFLP analysis, none of the six BC₁ progenies possessed all 12 individual tomato chromosomes (Table 3).

There were, obviously, discrepancies between the number of individual tomato chromosomes detected through GISH and those identified by RFLP analysis in all the BC₁ progenies, with the exception of 6739, in which the results of both methods were the same (Table 3). In each of the genotypes, 2002 and 2003, GISH detected 12 tomato chromosomes, whereas RFLP analysis identified only 11 and 10 chromosomes,



There were, however, discrepancies between the number of individual tomato chromosomes detected through GISH and those identified by RFLP analysis in all the BC₁ progenies with the exception of 6739, in which the results of both methods were the same (Table 2). In each of the genotypes 3002 and 5002, GISH detected 12 tomato chromosomes, whereas RFLP analysis identified only 11 and 10 chromosomes,

respectively. In genotype 2002, according to RFLP analysis, chromosome 3 was absent, and obviously, one among the 11 chromosomes identified was in duplicate. Similarly in 2003, although there were 12 alien tomato chromosomes, RFLP analysis detected only 10 individual chromosomes. This means that in this genotype two chromosomes were in duplicate and the rest were singles. An extreme case was that of 2004, in which, despite the presence of 11 tomato chromosomes, only 6 individual chromosomes were present and of these, five were in duplicate. Thus, from the use of a combination of GISH and RFLP analyses, the genome constitution and alien tomato chromosome composition in both the fusion hybrids and the six BC₁ progenies were established. One important fact that emerged from these analyses was that the available six BC₁ progenies were potentially useful for establishing of a complete series of tomato chromosome additions in a potato background.

Meiotic observations

Meiotic studies were confined to two fusion hybrids, C31-17-5 and C31-17-24, and one BC₁ progeny, 6739, in which microsporogenesis was investigated through GISH. The main aims were (i) to verify the genomic composition that had already been established through the examination of somatic chromosomes and RFLP analysis and (ii) to seek a probable explanation for the aneuploidy. In the fusion hybrid C31-17-24, GISH detected 23 tomato chromosomes and RFLP analyses detected all 12 different chromosomes of the complement (Table 3). Observations at metaphase I stages confirmed the presence of 11 bivalents and a univalent (Fig. 2B, arrow). Similarly, in the BC₁ progeny 6739, both GISH and RFLP analyses revealed the presence of 11

←

Fig. 2 Genome constitution and meiotic behaviour in potato (+) tomato fusion hybrids and their BC₁ progenies as revealed by GISH. In all the preparations, the tomato chromosomes fluoresce yellow-green because of the hybridization with total genomic DNA labelled with FITC (fluorescein isothiocyanate). The potato chromosomes are red owing to counterstaining with propidium iodide. **A** Somatic chromosomes of C31-17-51 ($2n = 6x - 4 = 68$) showing two translocated chromosomes (arrows). **B** Early metaphase I in C31-17-24 ($2n = 6x - 1 = 71$) showing 11 bivalents + 1 univalent (arrow) of tomato. **C** A later stage in metaphase I in C31-17-24 showing the precocious separation of tomato bivalents, while the potato bivalents are still held together. **D** Late anaphase I in C31-17-24 showing unequal separation of tomato chromosomes with one chromosome lagging (arrow). **E** Eleven univalents in BC₁ 6739 ($2n = 5x - 1 = 59$) at diakinesis (arrow indicates the large satellite of chromosome 2, accounting for a single one). **F** Somatic chromosomes of BC₁ 2001 ($2n = 5x - 3 = 57$) showing one translocated chromosome (arrow) and nine tomato chromosomes

tomato chromosomes, which was also confirmed in meiotic cells (Fig. 2E). Confirmation of the number of bivalents and univalents was all the more informative in BC₁ progeny plants, because it allowed us (i) to determine whether the chromosomes are in pairs, in which case they form bivalents or otherwise are univalents; and (ii) to establish whether the chromosomes possess any interchanges, in which case they might show association of potato and tomato chromosomes.

In view of the hexaploid chromosome constitution of the fusion hybrids in which there were two tomato genomes, the chromosomes were expected to pair as bivalents. This was indeed the case for all homologous chromosomes that were present as pairs in the fusion hybrids, especially during the early stages of metaphase I (Fig. 2B). From a cursory examination of many metaphase I stages, there was no indication of associations between potato and tomato chromosomes, i.e., allosyndetic pairing was absent. A notable feature was that the bivalents of tomato in the fusion hybrids fell apart as half-bivalents prematurely, compared with those of potato bivalents (Fig. 2C). As a result of this, the tomato chromosomes were distributed unequally during anaphase I stages, giving rise to aneuploid nuclei with lagging chromosomes (Fig. 2D). Thus, in addition to chromosome elimination, which was evident from aneuploidy in the fusion hybrids, non-synchronous separation of chromosomes during meiosis can also lead to aneuploidy in the subsequent generation. The abnormal meiotic behaviour of premature separation of tomato bivalents observed in microsporogenesis is expected to hold true for megasporogenesis as well.

In view of the improvement in pollen stainability of some of the BC₁ progenies (Table 2), they are expected to possess greater fertility than the fusion hybrids. Accordingly, three of the BC₁ plants, 6739, 2002, and 2003, were successfully used as female parents to produce BC₂ progenies.

Discussion

The first step in introgressing desirable alien chromosomes, or their parts, into a crop is to establish alien addition or substitution lines. The first backcross between the fusion hybrid C31-17-1 and potato (Jacobsen et al. 1994) generated optimism for the creation of potato lines with a complete series of monosomic tomato chromosome

additions. The single BC₁ plant that was obtained had retained only 6 of the possible 12 chromosomes (Jacobsen et al. 1995). Therefore, it was essential to produce more BC₁ plants in order to increase the potential for establishing a complete series of tomato addition-substitution lines in a potato background. In general, establishing BC₁ progenies by using the fusion hybrids between distant taxa within the Solanaceae is difficult. In most of the parental fusion combinations it was not possible to obtain seeds and BC₁ progeny because of poor growth in the glasshouse (E. Jacobsen, unpublished). In this sense, the discovery of the C31-17 series of potato (+) tomato fusion hybrids as suitable for backcrossing (Jacobsen et al. 1993) was a fortunate coincidence.

The six new BC₁ progenies produced and characterised in this investigation proved two points: (i) more BC₁ progenies can be obtained using different hexaploid fusion hybrids and different tetraploid pollen parents and (ii) these BC₁s are potentially useful for establishing a complete series of tomato addition-substitution lines in a potato background. Production of BC₁ progeny plants obtained in the present investigation (Table 1) needed much less labour compared with the first BC₁ plant reported earlier (Jacobsen et al. 1994), where only one of more than 4000 *in vitro* cultured ovules produced a single plantlet. This increase in efficiency was due to a more stringent selection of young seeds (developing ovules) at the proper developmental stage for *in vitro* culture, than to any genotypic effect or *in vitro* culture method.

The loss of the tomato chromosomes in the fusion hybrids C31-17-24 and C31-17-51 confirms some earlier observations (Shephard et al. 1983; Jacobsen et al. 1995). Should this type of chromosome elimination be as extreme as it is in the case of intergeneric hybrids of cereals (Riera-Lizarazu et al. 1996), for example, the task of establishing an addition-substitution series in potato would be difficult, if not impossible. Fortunately, there are instances where as many as 11 of the 12 individual tomato chromosomes have been retained in the fusion hybrids and sexually transmitted to the BC₁ progenies (e.g., 6739 and 2002, Table 3). Moreover, these BC₁s have been successfully used in further backcrosses to produce BC₂ progenies with alien tomato chromosome additions (Jacobsen et al. 1995; Garriga et al. in preparation). This

obviously indicates that a complete series of tomato chromosomes can be added to, or substituted in, potato lines.

For the purpose of transferring alien chromosomes across the boundaries of distantly related taxa within the Solanaceae, other approaches have been attempted, but there appears to be relatively little success: for example, asymmetric hybridization of protoplasts (Gleba et al. 1988; Schoenmakers et al. 1994; Samoylov et al. 1996) and microplasm fusion (Ramulu et al. 1994, 1996), in which finally the alien chromosomes seem to be eliminated (J.H. de Jong, personal communication). These techniques involve the treatment of donor protoplasts with either radiation or chemicals. The drastic effects of these treatments might damage the donor chromosomes to such an extent that they tend to be eliminated. In this context, the BC₁ and subsequent generations reported here and elsewhere (Jacobsen et al. 1995) are a step forward for transferring alien chromosomes across intergeneric barriers in the Solanaceae.

The identification of genomes and individual alien chromosomes using a combination of two different screening methods, viz., GISH and RFLP analyses, is a fruitful approach in plants with small chromosomes such as potato and tomato. Although GISH is a powerful method for detecting alien chromosomes, this approach alone is not sufficient for a rapid identification of individual chromosomes. RFLP analysis can identify the presence of particular individual chromosomes, but whether they are present as one of a pair or as single chromosomes cannot be determined. To confirm such a situation, GISH of meiotic preparations is exceptionally helpful (see also Jacobsen et al. (1995)). For chromosome identification, a single RFLP marker for each of the 12 different chromosomes has been used in the present study. For this reason, only one of the chromosome arms has been identified, but not necessarily the entire chromosome. To establish that a complete-chromosome addition is present, the use of multiple chromosome-specific probes together with meiotic studies is necessary. One puzzling observation was that two tomato chromosomes in the fusion hybrids appeared to possess translocated segments of potato chromosomes. Such a translocated chromosome was also observed in the backcross progeny as well (Table 2, Fig. 2F). A similar situation has been reported in the progeny of sexual hybrids of closely related *Solanum* species before (Wilkinson et al. 1995). If they are indeed

translocations, their mode of origin needs explaining. RFLP analysis of potato lines with such anomalous chromosomes, using several chromosome-specific probes, might resolve the question of whether they are translocations or not. Undoubtedly, translocated chromosomes, when present, would be attractive from the point of view of introgression.

3

Transmission of alien tomato chromosomes from BC₁ to BC₂ progenies derived from backcrossing potato (+) tomato fusion hybrids to potato: the selection of single additions for seven different tomato chromosomes

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Abstract

By backcrossing three BC₁ genotypes of potato (+) tomato fusion hybrids to different tetraploid potato pollinators, BC₂ populations were produced. A combined total of 97 BC₂ plants from three BC₂ populations were analysed with chromosome-specific probes through restriction fragment length polymorphism (RFLP) for the presence of alien tomato chromosomes. The number of different alien tomato chromosomes transmitted through the female BC₁ parent ranged from 0 to 6, and the average number of different alien chromosomes transmitted per BC₂ plant varied between 1.7 and 3.4 in the different populations. This variation corresponded to the chromosome constitution of the individual BC₁ parents: parent 6739, which possessed 11 different alien chromosomes in a single condition, gave rise to progeny with a lower average number of alien chromosomes per plant than the BC₁ parent 2003 that possessed 2 of the 12 alien chromosomes in a disomic condition. In the latter case, the higher transmission rate was attributed to the more regular distribution of the two alien chromosomes in the disomic condition because of regular bivalent formation during meiosis as revealed by genomic *in situ* hybridisation (GISH) and fluorescent *in situ* hybridisation (FISH). The transmission frequencies of individual alien chromosomes were subjected to statistical analysis to test whether the maternal genotypes had an effect on alien-chromosome transmission. Among the BC₂ plants, a total of 27 single additions were detected for as many as seven different chromosomes (1, 2, 4, 6, 8, 10 and 12) out of the 12 possible types.

Introduction

The transfer of alien chromosomes and genes across interspecific and intergeneric boundaries has been most useful in the past for crop improvement (Hadley and Openshaw 1980). Some of the notable examples of alien-chromosome transfer for crop improvement are the cereals (Jiang et al. 1994) and *Brassica* (This et al. 1990; Chevre et al. 1991; Struss et al. 1992). In the case of cereals, notable progress has been made with regard to yield, disease resistance and adaptation, through the introgression of alien segments of rye into wheat (Bartos 1993). In such cases, besides introgression, the alien chromosome additions also facilitated more accurate physical mapping of the genomes (Badaeva et al. 1995; Chen et al. 1995; Hohmann et al. 1995, 1996; Castilho et al. 1996). The process of creating such additions, however, is generally laborious and time-consuming. This problem can be especially serious when the involved parents are distantly related, as in the case of potato and tomato that belong to two different genera, viz., *Solanum* and *Lycopersicon* respectively. In addition to the crossing barriers, a host of other problems such as the difficulty of backcrossing the hybrid to the parents, the selective elimination of the alien chromosomes in the hybrids and in the BC progenies, as well as the non- or low-transmission of individual alien chromosomes through the gametes, are only some among numerous other bottlenecks.

The first successful production of somatic hybrids between potato and tomato (Melchers et al. 1978) opened up the possibility of transferring chromosomes and genes between these two important crop plants within the family Solanaceae. Nevertheless, until recently, the fusion hybrid could not be easily backcrossed to any of the parents. However, by crossing a hexaploid fusion hybrid ($2n = 6x = 72$) with potato ($2n = 4x = 48$), a single BC₁ plant was obtained (Jacobsen et al. 1994). This plant was found to possess only 6 of the expected 12 alien tomato chromosomes (Jacobsen et al. 1995). The absence of several of the tomato chromosomes in the BC₁ was either due to the selective chromosome elimination of somatic chromosomes, as reported in potato (+) tomato fusion hybrids (Shepard et al. 1983), or due to meiotic elimination in the BC progenies, as reported by Jacobsen et al. (1995). Because of this drawback, it was essential to produce more BC₁ plants and to evaluate whether all

alien tomato chromosomes were present and can be introduced into potato. To this end, new BC₁ progenies were produced by using additional individual fusion hybrids and potato pollinators (Garriga-Calderé et al. 1997). RFLP analyses of these BC₁s showed that all the tomato chromosomes were present at the BC₁ level. In addition, these new BC₁ progenies were successfully backcrossed to several tetraploid genotypes of potato and BC₂ progeny populations were obtained.

The aims of the present investigation were: (i) to characterise BC₂ populations in which aneuploids had originated from the sexual transmission of alien tomato chromosomes; (ii) to analyse the probability of female transmission of the alien tomato chromosomes from different BC₁s to BC₂ progeny plants, and (iii) to select potato genotypes with single additions of tomato chromosomes.

Materials and methods

Three BC₁ genotypes, 6739, 2002 and 2003 (Garriga-Calderé et al. 1997), derived from hexaploid potato (+) tomato somatic hybrids of the series C31-17- (see Jacobsen et al. 1994), were used as female parents. These BC₁s were successfully crossed with eight different tetraploid potato pollen parents, viz., a breeding clone AM66.42, cv Desirée, cv Katahdin, and five amylose-free starch-containing genotypes: 6704-1, 6704-6, 6706-1, 6706-2 and 6707-7 (Jacobsen et al. 1989). Corresponding to the three BC₁ female parents, three sets of populations of BC₂ progenies were analysed and the details of their origin are illustrated in Fig. 1. The sets of three BC₂ populations are grouped according to the female parents and, as indicated, will be referred as 1, 2 and 3.

The plant material, including the fusion parents, fusion hybrids, BC₁ plants, pollinators and the BC₂ progenies, were grown in the greenhouse under uniform environmental conditions. The genome constitution of the two fusion hybrids (C31-17-5 and C31-17-24), as well as of the three different BC₁s used was determined through GISH and RFLP analyses (Garriga-Calderé et al. 1997). Briefly, these analyses confirmed the presence of 11 different alien tomato chromosomes in BC₁ 6739 (chromosome 7 was absent). Chromosomes 5 and 12 were not detected by RFLP analyses in BC₁ 2003, although GISH revealed 12 tomato chromosomes indicating

Fusion	pollinator	BC ₁	pollinator	BC ₂	population
C31-17-24 (2n=6x=72-1)	X AM66.42 (2n=4x=48)	→ 6739 (2n=5x=48P+11T) (11 single + 0 dis.)	X 6706-1	→ 2101-1/8	1
			X 6704-6	→ 2102-1/10	
			X AM66.42	→ 2103-1/11	
C31-17-5 (2n=6x=72)	X 6704-1 (2n=4x=48)	→ 2003 (2n=5x=48P+12T) (8 single + 2 dis.)	X AM66.42	→ 2401-1/4	2
			X 6704-1	→ 2402-1/7	
			X 6707-7	→ 2403-1/14	
C31-17-5 (2n=6x=72)	X AM66.42 (2n=4x=48)	→ 2002 (2n=5x=48P+12T) (10 single + 1 dis.)	X Desiree	→ 2301-1/25	3
			X Katahdin	→ 2302-1/8	
			X 6706-2	→ 2303-1/10	

Fig. 1 Pedigree chart of the potato (+) tomato fusion hybrids and backcross progenies. Eleven alien tomato chromosomes were detected in a single condition in the BC₁ 6739, whereas one and two alien tomato chromosomes were detected in a disomic condition in BC₁s 2002 and 2003, as indicated by *1 dis.* and *2 dis.* respectively which regularly formed bivalents at meiosis. The column BC₂ indicates the code and number of plants in each BC₂ population

that two alien chromosomes were in a disomic condition and that they regularly formed two bivalents at metaphase I of meiosis (see Fig. 3). Likewise, chromosome 3 was not detected through RFLP analysis in BC₁ 2002 although GISH again revealed 12 tomato chromosomes. In this case, one alien tomato chromosome was in a disomic condition and regularly formed one bivalent at the metaphase I stage of meiosis (data not shown).

After backcrossing the BC₁s to tetraploid potato, the BC₂ populations were created by ovule culture as described by Jacobsen et al. (1993).

RFLP analysis

For RFLP analysis DNA from young shoot tips and leaves was extracted according to Bernatsky and Tanksley (1986). The procedures for DNA digestion and Southern

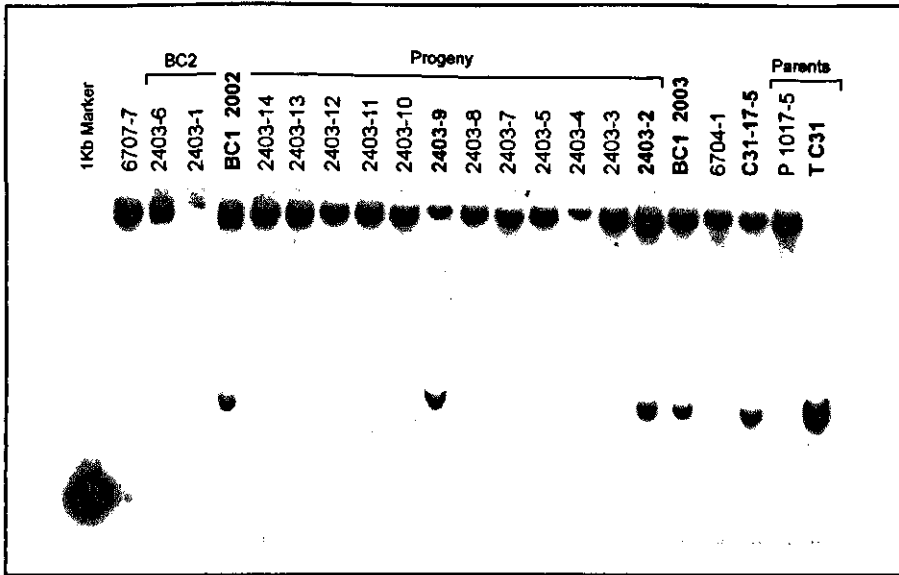


Fig. 2 A representative autoradiogram of a Southern blot illustrating the detection of an alien tomato chromosome addition through RFLP analysis. The tomato parent T C31 and the potato parent P1017-5 show clear polymorphism. Both bands are clearly present in the fusion hybrid C31-17-5 and in the two BC₁s, 2003 and 2002. The pollen parents, 6704-1 and 6707-7, clearly lack the polymorphic band observed in the tomato parent C31. In the 14 BC₂ progenies only two genotypes, 2403-2 and 2403-9, possess the polymorphic band of the probe TG46 which identifies chromosome 11 of tomato

hybridisation were according to Kreike et al. (1990). Polymorphisms were found after the DNA was digested with either *EcoRI* or *EcoRV*. For a reliable chromosome identification, the tomato chromosome-specific probes were selected on the basis that: (i) the probe showed clear polymorphism between the tomato and potato parents; (ii) the polymorphic bands of both species were clearly identifiable in the fusion hybrid; (iii) the polymorphic band of tomato was absent in the pollen parent(s) used in backcrosses, and (iv) the polymorphic band unequivocally identified the particular tomato chromosome in the BC₁ and BC₂ progenies (see Fig. 2).

The corresponding 12 chromosome-specific probes of tomato, in the order 1-12 were as follows: TG53; TG34; TG130; TG500; TG23; TG115; TG143; TG160; TG8; TG285; TG46; TG28. These probes were kindly provided by Prof. S.D. Tanksley, Cornell University, N.Y., USA.

GISH

The genomic constitution and the behaviour of the BC₁s used as female parents were studied through GISH. For that purpose, pollen mother cells from young flower buds were monitored for their stage of development. One anther of each flower bud was squashed in a drop of aceto-carmin and examined under the light microscope. The remaining anthers were fixed in ethanol-acetic acid (3:1). After 1/2 h fixation the anthers were rinsed three times for 10 min with 10 mM citrate buffer, pH 4.5. The material was then incubated in a pectolytic enzyme mixture consisting of 0.5% pectolyase Y23, 0.5% cellulase RS and 0.5% cytohelicase in 10 mM citrate buffer, pH 4.5, at 37°C for 2 h. Chromosome spreads on a grease-free slide were done according to Pijnaker and Ferwerda (1984). The protocol to perform *in situ* hybridisation was similar to that described by Schwarzacher and Heslop-Harrison (1994).

Total genomic tomato DNA was sonicated to a fragment size of 5-10 Kb and either directly labelled with fluorescein-11-dUTP or labelled with digoxigenin following a standard nick-translation protocol (Boehringer Mannheim). The potato DNA used for blocking was autoclaved for 5 min giving a fragment size of 200 - 500 bp. The hybridisation mixture, hybridisation conditions, stringency washing and counterstaining procedures were the same as those previously described by Garriga-Calderé et al. (1997).

FISH

In order to establish that one of the tomato bivalents in BC₁ 2003, was indeed chromosome 2 (the satellite chromosome) some of the slides hybridised with GISH were re-probed with rDNA (pTA 71) as follows: the cover slips, antifade, and the probe were removed by washing the slides 4 times for 1 h in 4 xSSC plus 0.5% tween 20. The slides were then dehydrated in an ethanol series.

The rDNA probe was pTA71 which contains the 5.8s-18s-26s ribosomal genes (Gerlach and Bedbrook 1979) and which was labelled with biotin-16-dUTP following a standard nick-translation protocol (Boehringer Mannheim). The hybridisation mixture, hybridisation conditions and stringency washings were similar to those mentioned above. The three detection steps were as follows; 4 µg/mL streptavidin-Cy3 (Jackson Immuno Research Laboratories), 10 µg/mL biotinylated-anti-

streptavidin (Vector Laboratories) and again 4 µg/mL streptavidin-Cy3. For each detection step the slides were incubated with 100 µL blocking buffer 1 (0.5% blocking reagent (Boehringer Mannheim) in buffer 1 (0.1M Tris-HCl plus 0.15 M NaCl, pH 7.0)) for 30 min, incubated for 1 h with the appropriated antibody in 100 µL blocking buffer, and washed 3 times in buffer 1 for 10 min at room temperature. The chromosome spreads were then counterstained with 2 µg/mL DAPI (4'6-diamidino-2-phenylindole) and mounted in 10 µL Vectashield (Vector Laboratories). Selected chromosome spreads were photographed on 400 iso colour negative film with an Axiophot microscope equipped with UV light and the appropriate filter block. Negatives were scanned at 300 dpi and the digital images were optimised for contrast and brightness using a routine image processing software.

Statistical analysis of alien tomato-chromosome transmission

The frequencies of female transmission within and among the BC₂ populations were statistically analysed. In the two analyses, chi-square tests were performed. The null-hypothesis that was used in each of the tests is indicated in Table 3. The level of significance that was chosen in each of these tests was $\alpha = 0.05$.

Results

Alien chromosome constitution of the BC₂ progenies

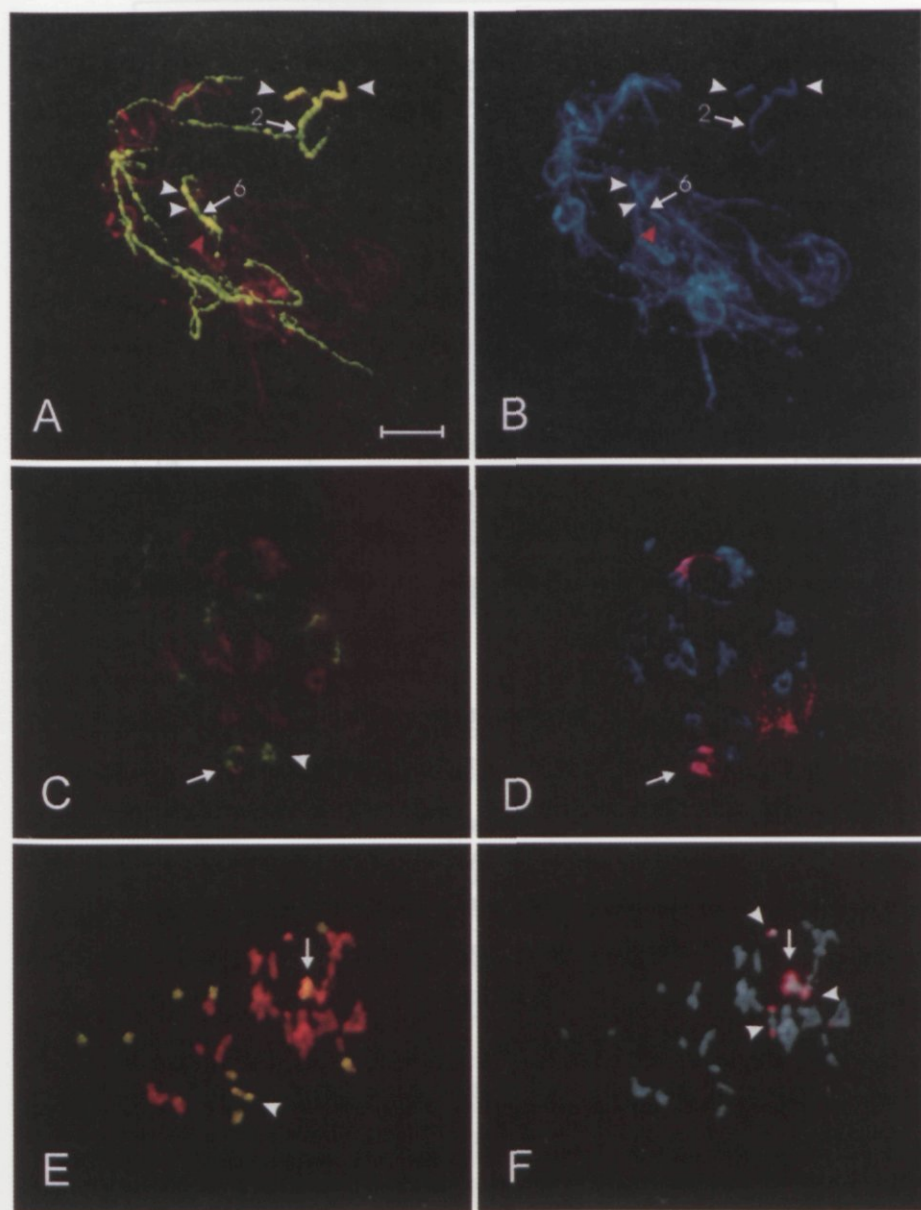
A total of 97 plants, belonging to three different BC₂ populations (Fig. 1), were evaluated for the presence or absence of alien tomato chromosomes through RFLP analysis. These three populations were derived from BC₁s that had different chromosome constitutions with regard to the number and type of individual tomato chromosomes (Fig. 1). The ploidy status and the number of alien chromosomes in the parental BC₁s, as well as in the BC₂ progenies, was that there were four intact genomes of potato together with a variable number of alien tomato chromosomes. The BC₁ parent 6739 possessed 11 single alien tomato chromosomes in contrast to BC₁s 2003 and 2002. BC₁ 2003 possessed eight alien tomato chromosomes in a single condition and two in a disomic condition, which regularly formed two bivalents at the metaphase I stage of meiosis (Fig. 3); whereas BC₁ 2002 possessed ten alien tomato

chromosomes in a single condition and one in a disomic condition, which regularly formed one bivalent at meiosis. The formation of bivalents during meiosis for those chromosomes present in a disomic condition in BC₁ 2003 and 2002 was constantly observed at different stages of meiosis. Based on their morphology, the bivalents could be unequivocally identified at the pachytene stage of meiosis (Figs. 3 A, B). In addition the bivalents were also detected at the diakinesis and metaphase I stages of meiosis (Figs. 3 C, E). In the latter two cases the formation of the chromosome 2 bivalent (satellite chromosome) was confirmed by FISH after re-probing the chromosome spreads with rDNA (Figs. 3 D, E).

Through repeated testing with RFLP probes (sometimes two) for a single chromosome it was established that identification of additional alien tomato chromosomes was completely reliable (Fig. 2). Based on such analyses, the alien chromosome constitution of the BC₂ progenies that were characterised are presented in Table 1.

The number and frequency of BC₂ plants to which one or more individual alien tomato chromosomes was transmitted differed within and among populations (Table 1). For example, 20.7% (5 out of 29 plants) of the BC₂ plants did not possess a single tomato chromosome (Fig. 4) in population 1. There was also variation for the rate of transmission of individual chromosomes. For example, 12 out of 29 plants (41.4%) possessed tomato chromosome 1 and not a single BC₂ plant possessed tomato chromosome 9. This means that among the 11 individual alien chromosomes of the

Fig. 3 Alien tomato chromosome constitution in BC₁ 2003, established through GISH and FISH, to determine univalents and bivalents. In the panels A, C and E the tomato chromosomes fluoresce green due to FITC labelling during GISH, whereas the potato chromosomes are red because of counterstaining with propidium iodide. A, B The same cell at the pachytene stage after GISH- and DAPI-staining respectively. Besides eight univalents (*thin structures*), two bivalents (*thick, arrowed*) of chromosome 2 and chromosome 6 can be identified morphologically. Centromere position and the heterochromatic regions of both chromosomes are marked with *arrows and arrow heads* respectively. The heterochromatic part of 6S, which is diagnostic for identification, is indicated with a *red arrow head*, and the two satellite parts of chromosome 2 are indicated with *white arrow heads*. C, D GISH and FISH images of the same cell in diakinesis confirming the bivalent of chromosome 2 (arrow) through hybridization, with pTA71 (5.8s-18s-26s) rDNA fluorescing red in D (arrow); arrow head in C corresponds to a ring bivalent of chromosome 6. E, F metaphase I stage used for both GISH and FISH to confirm the association of the nucleolar chromosomes using pTA71 (5.8s-18s-26s) rDNA as a probe; the arrow indicates the single tomato bivalent of chromosome 2 whereas the arrow heads indicate a bivalent (*small arrow head*) and two univalents (large arrow heads) of nucleolar chromosomes of potato. All figures are of the same magnification and the bar (A) represents 10 μm



Transmission of alien chromosomes to BC₂ progeny was 3.1 per plant. The very high transmission rate of alien chromosomes was observed in the BC₂ population. The transmission rate of chromosomes 3 and 6 (91.0 and 88.0% respectively) in the BC₂ population was similar to that observed in the BC₁ population. The transmission rate of chromosome 3 and of chromosome 6 (88.4%) in population 3 are assumed to be due to the presence of a disomic condition in the BC₂ population. Chromosomes 3 and 6 are expected to form bivalents during meiosis in the BC₂ population. The presence of a disomic condition in the BC₂ population should lead to their more regular transmission (Lopatin et al. 1997) which should lead to their more regular transmission to the BC₂ progeny. As shown in Fig. 4, the more regular transmission of chromosomes 3 and 6 to the BC₂ progeny is reflected in the high transmission rate of these chromosomes in the BC₂ population.

Table 1 Number and frequency of transmission of individual alien tomato chromosomes in three different BC₂ populations

Chromosome	Population 1 (29 plants)		Population 2 (25 plants)		Population 3 (43 plants)	
	n plants	Frequency %	n plants	Frequency %	n plants	Frequency %
1	12	41.4	2	8.0	7	16.2
2	5	17.2	23	92.0	3	6.9
3	1	3.4	7	28.0	-	-
4	7	24.1	7	28.0	3	6.9
5	3	10.3	-	-	5	11.6
6	4	13.8	22	88.0	38	88.4
7	- ^a	-	1	4.0	6	13.9
8	7	24.1	9	36.0	9	20.9
9	0	0.0 ^b	8	32.0	3	6.9
10	3	10.3	5	20.0	5	11.6
11	4	13.8	2	8.0	4	9.3
12	4	13.8	-	-	8	18.6
Transmission per plant	1.7		3.4		2.1	
BC₁^c	11 single + no disomics		8 single + 2 disomics		10 single + 1 disomic	

^a The alien tomato chromosome was already absent in the BC₁

^b 0 = not transmitted

^c Alien chromosome constitution based on RFLP and GISH analyses (Garriga-Calderé et al. 1997)

BC₁ parent 6739, only ten were transmitted to individual plants of this BC₂ population. The average number of transmissions of tomato chromosomes to BC₂ plants in this population was 1.7. In the other two BC₂ populations, the range of transmission of particular alien chromosomes was even more extreme than that found for chromosomes 1 and 9 in population 1. In contrast with population 1, all the BC₂ plants possessed alien tomato chromosomes in population 2, and the transmission rate in this population varied between 4.0 and 92.0% for chromosomes 7 and 2 respectively. The average transmission of alien chromosomes to BC₂ progeny was 3.4 per plant (Table 1). On the other hand, only one plant did not possess a single alien tomato chromosome in population 3 and the transmission rates in this population varied between 6.9% for chromosomes 2, 4 and 9 to 88.4% for chromosome 6. In this population the average transmission of alien chromosomes to BC₂ progeny was 2.1 per plant. The very high transmission rates of chromosomes 2 and 6 (92.0 and 88.0% respectively) in population 2, and of chromosome 6 (88.4%) in population 3, are assumed to be due to their presence in a disomic condition in the respective BC₁ plants. Chromosomes present in a disomic condition in the BC₁s are expected to form bivalents during meiosis (Jacobsen et al. 1995) which should lead to their more regular

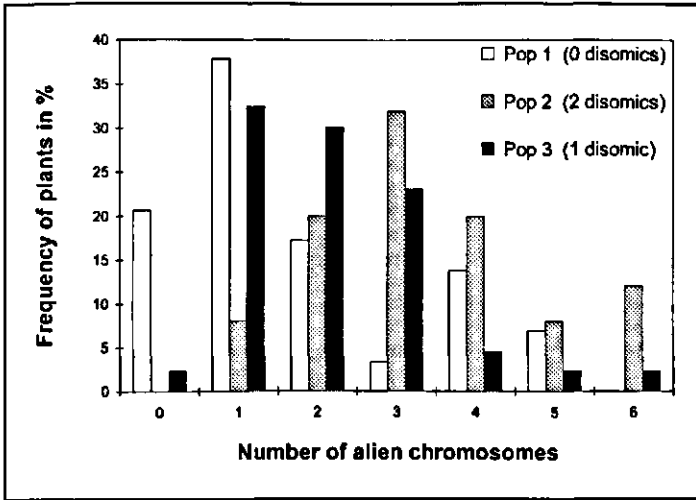


Fig. 4 Histogram of the frequency of classes for three different BC₂ populations in relation to the number of alien tomato chromosome additions per BC₂ plant. Note that there is a shift towards a higher number of chromosome additions when more chromosomes in a disomic condition (as indicated with 0, 1 and 2 disomics) were present in the BC₁ parent

distribution to the gametes and, consequently, to a higher frequency in their progeny, as indeed proved to be the case. However, when the transmission rate of those chromosomes that regularly formed bivalents at meiosis (chromosome 2 and 6 in population 2, and chromosome 6 in population 3) was excluded, the average transmission per plant appeared to be fairly constant among the three BC₂ populations. The average number of alien tomato chromosomes per plant was 1.7 as indicated for population 1, 1.6 for population 2, and 1.2 for population 3.

The number of alien chromosomes present in individual BC₂ plants ranged from zero to six (Fig. 4). The frequency of plants with a different number of alien chromosomes varied among the three populations. A notable feature was that the frequency of BC₂ plants with different numbers of individual tomato chromosomes clearly reflected the chromosome constitution of the BC₁ parents. In other words, it depended on whether the BC₁ parent possessed only chromosomes in a single condition as in BC₁ 6739, the parent of population 1, or had one or two of the alien chromosomes in a disomic condition so forming bivalents at meiosis. The last mentioned situation was found in BC₁ 2002, the parent of population 3, and in BC₁ 2003, the parent of population 2. As shown in Fig. 4, the more-representative classes

in population 1 appeared to possess between 0 and 2 tomato alien chromosomes. Due to the presence of 11 individual chromosomes in BC₁ 6739, more than 20% of the progeny plants did not possess alien chromosomes. In this case the frequency of the single addition genotypes selected was the highest among the three BC₂ populations (37.9%, Table 2). On the other hand, in population 3 almost all plants (except one) contained alien tomato chromosomes and the more frequent classes possessed between one and three additional ones (Fig. 4). In population 2, all the plants possessed alien chromosome additions and the majority of the plants fell into the classes with 2-4 additional alien tomato chromosomes. Therefore, the BC₂ populations showed a progressive shift to a higher number of alien tomato chromosome additions when an increasing number of alien tomato chromosomes in a disomic condition (forming bivalents at meiosis) were present in the BC₁ parents. Thus, in view of selecting plants with only one alien tomato chromosome addition, populations 1 (from a BC₁ with no disomics) and 3 (from a BC₁ with one disomic, forming one bivalent at meiosis) were preferable.

The single tomato chromosome additions that were selected are shown in Table 2. These single additions were the most preferred among the aneuploids of the three populations investigated. A total of 27 single additions were detected in the three populations. As indicated earlier, there were clear differences among the three populations with regard to the frequencies of single additions recovered. The highest frequency of single additions (37.9%) was found in population 1 (Table 2). In this case, as many as six different single additions could be selected. Next came population 3, where 32.5% of the plants possessed single additions. Nevertheless, these consisted of single additions for only three different chromosomes, viz., 6, 8 and 12. Remarkably, 78.6% of these single three different chromosomes, viz., 6, 8 and 12. Remarkably, 78.6% of these single additions were found to be for chromosome 6, which is clearly related to its presence in a disomic condition thus forming bivalents at meiosis in the BC₁ parent. The lowest number of single additions (8%) were detected in population 2. They were only found for two different chromosomes, viz., 2 and 6, which, once again, were those in a disomic condition and which regularly formed two bivalents at meiosis (Fig. 3) in the BC₁ parent.

Table 2 Frequency and types of single^a additions of individual tomato chromosomes found in the three different BC₂ populations based on RFLP analyses

BC ₁ constitution	BC ₂ Pop.	Alien tomato chromosome in single additions											Total	%	
		1	2	3	4	5	6	7	8	9	10	11			12
11 single + 0 disomics	1	3	2	0	3	0	1	0	0	0	1	0	1	11	37.9
8 single + 2 disomics	2	0	1	0	0	0	1	0	0	0	0	0	0	2	8.0
10 single + 1 disomic	3	0	0	0	0	0	11	0	2	0	0	0	1	14	32.5

^aThey could be in a mono- or di-somic condition

Probability of the female transmission of individual alien tomato chromosomes

(i) Within the BC₂ populations.

The null-hypothesis that the individual alien chromosomes were transmitted with equal probability was rejected in all three populations (Table 3). This observation indicated that there were systematic differences among individual alien tomato chromosomes with regard to female transmission. This was partly expected because of the presence of the alien tomato chromosomes in a disomic condition, giving rise to bivalents at meiosis. Therefore, when the null-hypothesis was tested, after excluding those chromosomes in a disomic condition in the BC₁s (chromosomes 2 and 6 for population 2, and chromosome 6 for population 3), an unequal probability of transmission could not be proven for the remaining chromosomes of these two populations. Likewise, when the composition of population 1 was carefully examined and the test was performed by excluding the more deviating chromosome (1), the null-hypothesis of an equal probability of transmission for the remaining ten chromosomes could not be rejected ($P = 0.898$).

(ii) Among the BC₂ populations

In this case, only those chromosomes that were present in a single condition in all three BC₁ parents were employed to test the null-hypothesis of an equal probability of transmission (Table 3, see also Table 1). For the alien tomato chromosomes 1 and 9 the null-hypothesis was rejected, whereas an unequal probability of chromosome transmission among populations could not be proven for the remaining four (chromosome 4, 8, 10 and 11).

Table 3 Testing for equality of female transmission of the different alien tomato chromosomes. In the case of disomics in BC₁, the test of equal transmission in the population 2 and 3 calculations were made by both including and excluding the disomic tomato chromosomes

Within the three BC ₂ populations					
Disomics in BC ₁	Population	n classes ^a	χ^2 ^b	df ^c	P ^d
0	1	11	23.56	10	0.008
2	2	10	65.21	9	0.000
0	2	8	12.37	7	0.089
1	3	11	122.63	10	0.000
0	3	10	7.94	9	0.540
Among the three BC ₂ populations					
1 ^e		3	7.72	2	0.021
4 ^e		3	3.75	2	0.153
8 ^e		3	0.33	2	0.847
9 ^e		3	8.78	2	0.012
10 ^e		3	0.23	2	0.891
11 ^e		3	0.51	2	0.774

^a n classes; number of chromosomes used in the test (within) and number of populations used in the test (among)

^b χ^2 , outcome of the statistical test

^c df, degrees of freedom

^d P, the significance probability

^e individual chromosome tested

Discussion

Generally, alien chromosomes tend to be eliminated preferentially in distant somatic hybrids and in BC progenies made within the Solanaceae family. For example, tomato chromosomes were reported to be somatically eliminated preferentially in potato (+) tomato fusion hybrids (Shepard et al. 1983) and meiotically in the BC progenies (Jacobsen et al. 1995). Despite these difficulties, the present investigation showed that it is possible to establish a large number of potato genotypes with alien tomato chromosome additions. In fact, out of the 97 BC₂ plants that were investigated, a range of alien chromosome additions, from 1 to 6, was found to be present in 90 plants (92.8%). This is a strong indication that the potato genotypes not only retain the alien chromosomes somatically in a stable condition in a substantial number of BC₂ progenies, but also that the BC₁ parents can transmit them efficiently through the female gametes to the progeny. However, the way in which this material was obtained needs to be considered. Both BC₁ and BC₂ progenies had to be created through embryo-rescue techniques (see Chap. 1, Fig. 1, pag. 6) due to their failure to develop

into mature seeds normally. By in vitro culture, the young embryo is somehow forced to develop in a special culture medium. As a result of this, it is likely that the elimination of embryos hosting the alien tomato chromosomes is prevented. This might explain the relatively high frequency of BC₂ plants (92.8%) possessing alien chromosome additions.

There were indeed differences among the 12 individual tomato chromosomes that were transmitted to the BC₂ progenies within and among the three populations. Within population 1, for example, chromosome 1 of tomato was present in the largest number of plants (41.4%) and not a single plant contained chromosome 9 (Table 1). This trend was, fortunately, reversed in population 2 in which a substantial percentage (32.0%, Table 1) of plants possessed chromosome 9 of tomato. Such variation in the transmission of individual alien chromosomes probably reflects genotypic differences among the female BC₁ parents of the three populations. Similar differences with regard to the transmission of individual extra chromosomes in different backcross combinations within a species have been reported in an investigation of tomato trisomics (Khush 1973). In addition, this type of difference was also reported in intergeneric combinations in the production of alien chromosome additions of *Brassica nigra* in a *Diplotaxis erucoides* background (This et al. 1990).

A much higher frequency of alien chromosome transmission was observed for those alien tomato chromosomes that were present in a disomic condition and which regularly formed bivalents during meiosis in the BC₁s (e.g. chromosomes 2 and 6 for population 2, and chromosome 6 for population 3). The same phenomenon was found to occur in a BC₂ population earlier described by Jacobsen et al. (1995). This does not, however, mean that the presence in a disomic condition was solely responsible for the higher frequency of transmission. In the case of chromosome 1 in population 1, despite being represented as a single copy, its transmission was 41.4%, in contrast with 0.0-24.1% for the other single chromosomes. This was either due to the effect of the BC₁ genotype or to a higher rate of survival of spores, gametes, or zygotes possessing this alien chromosome 1 of tomato.

Considering the transmission of individual alien tomato chromosomes across populations, only two of them, viz., 1 and 9, among the six tested, viz., 1, 4, 8, 9, 10 and 11, deviated from an equal probability of transmission. This could be due to the

fact that the transmission of these two chromosomes was more deviating in population 1 (Table 1). On the other hand, statistical differences in the transmission rate could not be proved for the remaining four chromosomes, viz., 4, 8, 10 and 11.

The number of alien chromosomes transmitted from the BC₁ parents is an important consideration. If several chromosomes are transmitted through each gamete, this gives rise to progeny with mainly multiple chromosome additions rather than to single ones. In this respect, population 1 was the most preferable given the fact that fewer chromosomes per plant (1.7/plant, see Table 1 and Fig. 4) were transmitted. Unlike the situation in population 1, a higher average number of alien chromosomes was transmitted in the case of populations 2 and 3 (3.4 and 2.1 per plant respectively), which was less desirable for selecting single additions for different chromosomes. In keeping with this observation, six different types of single additions (chromosomes 1, 2, 4, 6, 10 and 12) were recovered in population 1, but only two (chromosomes 2 and 6) in population 2 and only three (chromosomes 6, 8 and 12) in population 3. The explanation for these differences lies in the fact that in BC₁ 6739, 11 individual tomato chromosomes were present, as earlier detected by GISH and RFLP analysis (Garriga-Calderé et al. 1997). These alien chromosomes are distributed irregularly during anaphase I of meiosis and, therefore, relatively fewer chromosomes are included in some of the gametes. On the other hand, the other two BC₁s, 2002 and 2003, possessing one or two alien chromosomes in a disomic condition have the possibility of regularly forming one or two bivalents respectively. The distribution of these chromosomes in a disomic condition is expected to be more normal during meiosis. Therefore, in these two cases one-to-several alien chromosomes are expected to be included in the gametes. This clearly explains why the BC₂ progenies of populations 2 and 3 possessed a higher number of alien chromosomes than the BC₂ progenies of population 1 (Fig. 4). The importance of chromosome pairing for chromosome transmission has also been well established in the case of maize (Einset 1943). In the present investigation, the high frequencies of transmission of chromosomes 2 and 6 in population 2, and chromosome 6 in population 3, can be attributed to the fact that they formed bivalents regularly (Fig. 3). Similarly, a high transmission rate of the alien tomato chromosome 6 was earlier reported by Jacobsen et al. (1995) during the creation of a different BC₂ population using related material.

Although 27 presumed single additions were detected, not all of them are likely to be genuine monosomics. For example, one of the individuals that was identified as monosomic for chromosome 10 in population 1 turned out to be a disomic addition after additional GISH analyses. Similar observations were earlier reported in another BC₂ population of a potato (+) tomato hybrid (Jacobsen et al. 1995). Additional GISH analyses are needed to discriminate between monosomic and disomic additions in the BC₂ progenies. The mechanisms that may operate in the formation of disomic additions will be discussed elsewhere (Garriga-Calderé et al. in preparation). The occurrence of such spontaneous disomics for individual alien chromosomes is clearly an advantage since this saves the laborious task of selfing or intercrossing true monosomics in order to select disomics. Comparable to this, Quiros et al. (1988) also recovered disomic additions in a second-backcross generation for the production of single additions of *B. nigra* chromosomes into *D. erucoides*. Such disomic additions are equally, or even more, useful for genetic analysis, maintenance and introgression. When disomic conditions for any of the chromosomes are identified, it would be relatively easy to generate progenies with monosomic additions from such plants.

Of the 27 genotypes of single additions that have been selected so far (Table 2), as many as 7 of the 12 possible types of the complete series have been recovered. The remaining five were absent either due to the low transmission rate of these particular chromosomes or because of the non-survival of the spores, gametes, or sporophytes possessing them as extra chromosomes individually.

The creation of alien addition lines can be most useful for various purposes as has been mainly demonstrated in cereals (see Jiang et al. 1994) and, to a lesser extent, in *Brassica* (This et al. 1990; Chevre et al. 1991; Struss et al. 1992). In the first place, mono- and di-somic additions form the first important step in the process of introgression. Secondly, single chromosome additions into an alien genetic background can facilitate more accurate physical and genetic mapping of the individual chromosomes. In this regard, several examples have been reported in cereals (Badaeva et al. 1995; Chen et al. 1995; Hohmann et al. 1995, 1996; Castilho et al. 1996). Thirdly, alien chromosome additions can be useful for the assessment of the phenotypes of some of the genes, as has been demonstrated for the gene(s) conferring blackleg disease resistance from *B. nigra* in the background of *B. napus* (Chevre et al.

1996). In this context, the alien tomato addition lines obtained in this investigation fulfil the first basic step. Once the potato lines with a complete series of alien tomato chromosome additions/substitutions are selected they can be used for a critical evaluation and for the localisation of characters determining resistances to biotic and abiotic factors, as has been demonstrated in the case of wheat-rye substitution lines (Bartos 1993).

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4

Origin of an alien disomic addition with an aberrant homologue of chromosome 10 of tomato and its meiotic behaviour in a potato background revealed through GISH

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Submitted for publication

Abstract

While characterising the potato (*Solanum tuberosum*, $2n = 4x = 48$) clones with alien tomato (*Lycopersicon esculentum*) chromosome additions, a single addition for chromosome 10 of tomato was identified through restriction fragment length polymorphism (RFLP) analysis. This plant, 2101-1, was a BC₂ derivative from a cross between a potato (+) tomato fusion hybrid backcrossed to potato. Cytological analysis of its somatic chromosomes through genomic *in situ* hybridisation (GISH) indicated the presence of four genomes of potato plus two alien tomato chromosomes of which one was much smaller than the other. Analysis of chromosome pairing at pachytene and metaphase I stages of microsporogenesis indicated that the large and small chromosomes were homologues. Thus, it was a disomic addition for chromosome 10 of tomato. The size difference was found to be due to a deletion. Fluorescent *in situ* hybridisation (FISH) experiments using the telomeric repeat pAtT4 from *Arabidopsis thaliana* and sub-telomeric sequences TGRI showed intact ends for both alien chromosomes. Thus, the deletion that the smaller of the homologues suffered was interstitial and most probably occurred in the centromeric heterochromatic region of the long arm. The pattern of distribution of large and small chromosomes to telophase II nuclei during microsporogenesis indicated that the deletion did not affect the meiotic behaviour of the smaller chromosome. In contrast, the frequencies of transmission of the large and the small chromosomes through the female parent, estimated in 96 BC₂ progeny of plants by RFLP and GISH analyses, appeared to be very different, 69.2 % and 3.8 % respectively. This study also provides evidence that two different chromatids of a pair of homologues rather than two chromatids of a single chromosome are most likely to be involved in the origin of a disomic. The aberrant chromosome can be used for physical mapping of the chromosome.

Introduction

Alien chromosome additions have been traditionally used for gene introgression in plants but they are also becoming increasingly useful in molecular cytogenetics. Some examples, among others, are: (i) to determine syntenic relationships and increase the accuracy of genetic maps (Chen et al. 1997; Suen et al. 1997), (ii) molecular tagging and cloning of alien genes (Ishii et al. 1994; Potz et al. 1996), (iii) physical mapping of chromosomes (Gill et al. 1996), (iv) to establish chromosome specific DNA libraries (Riera-Lizarazu et al. 1996; Ananiev et al. 1997), (v) introgression mapping (King et al. 1997), and (vi) to unravel the molecular organisation of individual, or parts of, chromosomes (Fransz et al. 1996; Zhong et al. 1996; H.W. Raines personal communication). Because of these attractive features, interest in distant hybrids and their backcross derivatives has increased in several crops such as cereals (review by Jiang and Gill 1994), solanaceous crops (Parokonny et al. 1992; Jacobsen et al. 1994; Suen et al. 1997), *Brassica* species (Quiros et al. 1988; Chen et al. 1997), rice (Multani et al. 1994), *Beta* species (van Geyt et al. 1988; Reamon-Ramos and Wricke 1992; Mesbah et al. 1996) and cotton (Ji et al. 1997).

Addition of whole or parts (recombinant) of chromosomes into an alien background can be useful for molecular cytogenetic purposes mentioned above. Furthermore, when chromosomes with aberrations, such as deletions and translocations, that can be assigned to specific chromosomes are added into an alien background, they can be highly attractive for physical mapping as has been demonstrated in bread wheat (Endo and Gill 1996; Gill et al. 1996). The chromosome aberrations in the wheat system are caused by the so-called gametocidal genes that are present in specific genotypes. For example, when the genome of *Aegilops cylindrica* is added into the bread wheat, large numbers of chromosome aberrations are induced by the gametocidal genes present in the species (Endo 1990). It is not known whether such possibilities exist in other plant systems, but the occurrence of chromosome aberrations in an alien background has often been observed in other plant hybrids as well, e.g. *Nicotiana* (Smith, 1968; Lin and Chen, 1990), and maize (Rhodes and Dempsy, 1966).

In our efforts to characterise alien tomato (*Lycopersicon esculentum*) chromosomes introgressed into potato (*Solanum tuberosum*) genotypes (Jacobsen et al. 1995; Garriga-Calderé et al. 1997, 1998), some instances of aberrations of alien chromosomes have been observed. Here we describe (i) the detection and characterisation of an interstitial deletion in chromosome 10 of tomato in a disomic addition derived from backcrosses of a potato (+) tomato fusion hybrid, (ii) the meiotic behaviour of the normal counterpart along with the mutant chromosome 10 of tomato in one and the same plant, and (iii) the rate of transmission of the normal and the mutant chromosomes.

Materials and methods

Plant material

The BC₂ plant 2101-1, a disomic addition possessing an aberrant homologue for the alien tomato chromosome 10, which was derived from a near hexaploid ($2n=6x=72-1$) tomato (+) potato somatic fusion hybrid repeatedly backcrossed to tetraploid ($2n=4x=48$) potato (Garriga-Calderé et al. 1998) was used to study alien chromosome distribution and chromatid assortment during microsporogenesis through GISH analysis.

A BC₃ population consisting of 96 plants created after backcrossing the 2101-1 plant to a nulliplex potato clone with amylose-free starch (*amf*), 6704-3 (Jacobsen et al. 1989), was used to study female transmission of the alien chromosome through RFLP and GISH analyses.

RFLP analysis

A BC₃ population consisting of 96 plants was analysed through RFLP for female transmission following the methodology of Kreike et al. (1990). Two DNA probes, TG285 and TG303, specifically identified the long and short arm respectively of chromosome 10 of tomato were used for the detection of the presence (or absence) of this chromosome. The probes were kindly provided by Prof. S.D. Tanksley, Cornell University, NY, U.S.A.

GISH and FISH analyses

For analysing the chromosome constitution in mitotic cells the root tips were harvested and treated according to Garriga-Calderé et al. (1997). For meiotic studies the young flower buds with suitable meiotic stages were used as previously described (Garriga-Calderé et al. 1998). Chromosome spreads on a grease-free slide were done according to Pijnaker and Ferwerda (1984). The protocol to perform genomic *in situ* hybridisation was similar to that described by Schwarzacher and Heslop-Harrison (1994). Tomato DNA was sonicated to a fragment size of 5-10 kb and labelled with digoxigenin-11-dUTP following a standard nick-translation protocol (Boehringer Mannheim). The hybridisation mixture, hybridisation conditions and stringency washing procedures were the same as those described previously (Garriga-Calderé et al. 1997). Digoxigenin was detected with 20 µg/mL anti-dig-FITC (fluorescein isothiocyanate); (Boehringer Mannheim). and 20 µg/mL rabbit-anti-sheep-FITC (Vector Laboratory). For each detection step the slides were incubated with 100 µL blocking buffer 1 (0.5% blocking reagent (Boehringer Mannheim) in buffer 1 (0.1M Tris-HCl plus 0.15 M NaCl, pH 7.0)) for 30 min, incubated for 1 h with the appropriated antibody in 100 µL blocking buffer, at 37°C, and washed 3 times in buffer 1 for 10 min at room temperature. The chromosome spreads were then counterstained with 5 µg/mL propidium iodide and with 2 µg/mL DAPI (4'-6-diamidino-2-phenylindole) for 10 min each at room temperature and mounted in 10 µL Vectashield (Vector Laboratories).

In order to determine the presence (or absence) of telomeres, the telomeric sequences of *Arabidopsis thaliana*, pAtT4 (Richards and Ausubel 1988), were used as a probe for FISH. The probe was labelled with biotin-16-dUTP as above. The washing of the probe, hybridisation mixture, conditions, stringency washings and detection with streptavidin-Cy3 procedures, were the same to those described previously (Garriga-Calderé et al. 1998). The telomeric probe was kindly provided by Dr. E.J. Richards, Washington University, MO, U.S.A. Besides telomeric probe, the sub-telomeric probe TGR1 was used for the confirmation of chromosome ends. TGR1 probe was kindly provided by Prof. S.D. Tanksley, Cornell University, NY, U.S.A.

Selected chromosome spreads were photographed on 400 iso colour negative film with an Axiophot microscope equipped with UV light and the appropriate filter

block. Negatives were scanned at 300 dpi and the digital images were optimised for contrast and brightness using a routine image processing software.

Results

Detection of alien additions

The disomic addition was detected in a BC₂ progeny, clone 2101-1, resulting from repeated backcrossing of a hexaploid potato (+) tomato somatic hybrid to the tetraploid potato as a male parent. Initially, detection of alien chromosomes in the progeny was based on RFLP analysis using a complete series of tomato chromosome-specific DNA probes, two per chromosome, in each case. In the clone 2101-1, RFLP analysis detected only the chromosome 10 of tomato. Because RFLP analysis alone could not discriminate between the presence of a complete vs. partial or a single vs. a pair of alien chromosome additions, this plant was further analysed through GISH using its somatic cells. Unexpectedly, instead of a single alien addition, a pair of tomato chromosomes was present in this plant in addition to the four genomes of potato. A striking feature was that one of the alien chromosomes was much smaller than the other in the somatic cells (Fig. 1A). In order to establish whether the smaller of the two alien chromosomes was a derivative of chromosome 10 of tomato, a detailed analysis of chromosome morphology and pairing was carried out during microsporogenesis.

Chromosome morphology and pairing

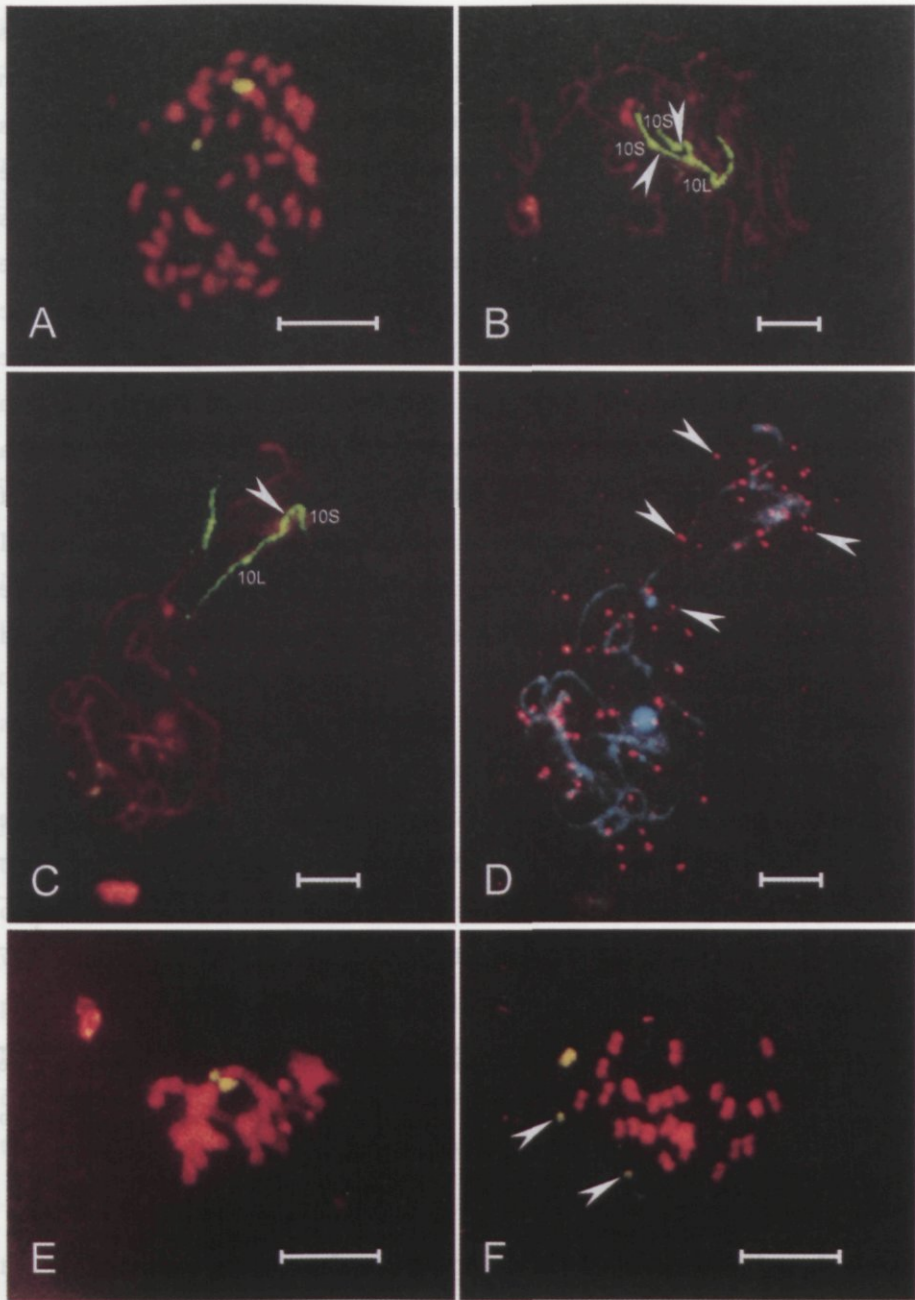
At pachytene stage the difference of size observed between the pair of alien chromosomes in somatic cells was confirmed (Fig. 1B and C). The morphology of the larger (intact) chromosome at pachytene stage (Fig. 1C) strictly conformed to the description of the pachytene chromosome 10 of tomato (Ramanna and Prakken 1967). The short arms of the bivalent were morphologically identical (Fig. 1B) whereas one of the long arms of the homologue had suffered a deletion. Despite the size differences, the two alien chromosomes paired as homologues to a great extent and occasionally with a loop formation. This was a clear indication that the smaller alien chromosome had originated from chromosome 10 of tomato, obviously through a

deletion. Granting that it was a deletion, the question arose whether it was due to a terminal or an interstitial loss in the chromosome. In order to answer that, the preparations in which both of the alien chromosomes were identified through GISH were reprobated using the telomeric sequence, pAtT4, for FISH analysis. Both of the alien chromosomes had all the telomeres intact in the occasional univalents at pachytene stage (Figs. 1C and D). In addition, the presence of the sub-telomeric sequence repeat TGR1 in both, the normal and mutant chromosomes was confirmed through FISH analysis (data not shown). The obvious conclusion was that the smaller chromosome had originated through an interstitial deletion in the long arm of chromosome 10. The considerable difference in the size of the homologues was probably due to a deletion of a substantial part of the proximal heterochromatic region on the long arm of chromosome 10. A more convincing proof that the large and the small alien chromosomes were indeed homologues was established from the fact that they regularly formed a heteromorphic bivalent (Fig. 1E) at metaphase I stage indicating chiasma formation.

Alien chromosome distribution

In view of pairing and chiasma formation, the two alien chromosomes were expected to disjoin more or less normally at anaphase I (Fig. 2A) and divide and distribute regularly to the four poles at anaphase II (Fig. 2C). Although such regular behaviour of the bivalent and half-bivalents was observed in some of the pollen mother cells,

Fig. 1 Chromosome constitution and meiotic behaviour of an alien chromosome pair in the BC₂ clone 2101-1. Except for **D** the alien tomato chromosomes fluoresce yellow due to FITC labelling of tomato genomic DNA whereas potato chromosomes fluoresce orange-red due to propidium iodide counterstain. Scale bars represent 10 μm. **A** Somatic chromosomes of the clone 2101-1 showing 48 potato chromosomes (*red*) and a pair of alien tomato chromosomes (*yellow-green*), one being much smaller than the other. **B** The pachytene stage showing a bivalent in which part of the long arms of chromosome 10 are synapsed, one being much smaller than the other, whereas the short arms are unpaired (*arrow heads* indicate the centromeres). **C** The pachytene stage showing two univalents of alien chromosomes one large (normal, the *arrow head* indicates centromere) and the small one. Note: the morphology of the large one conforms to chromosome 10 of tomato which is sub-median. The smaller chromosome, because of the fold-back pairing, has a different morphology in this cell. **D** Same cell as in **C** probed with telomeric repeat, pAtT4, through FISH (*red fluorescence*). Four telomeres corresponding to the two univalents are indicated by *arrow heads*. **E** Early metaphase I stage showing a heteromorphic bivalent of the alien pair of chromosomes. **F** Metaphase I stage showing precocious division of the smaller half-bivalent. The two chromatids are indicated by *arrow heads*



deviations were observed in many cases. These deviations consisted of precocious disjunction of bivalents at metaphase I followed by equational division of one or both half-bivalents at metaphase or anaphase I (Figs. 1F and 2B), lagging of half-bivalents as well as irregular distribution of chromatids at anaphase II stages (Fig. 2D). Because the irregular distribution of half-bivalents and chromatids during meiosis determines the composition of meiotic products, a quantitative estimate was made of the distribution of the large and small chromosomes during both first and second meiotic divisions in pollen mother cells (Table 1). From this study it was evident that in more than 60% of the pollen mother cells of both first and second divisions the alien chromosome distribution was abnormal. Such abnormalities included, among others, a premature equational separation of the alien chromosomes at anaphase I in 26.8% of the cases (Fig. 2B) what apparently lead to an irregular segregation to telophase II nuclei in 27.5% of the cases (Table 1, Fig. 2D). The lowest percentages of nuclei were those showing a normal segregation to four poles, 12.5 and 11.2 % at telophase I and II respectively (Table 1, Figs. 2A and C).

Table 1 Distribution of alien chromosomes (large and small) to the poles at anaphase I and anaphase II stages of microsporogenesis

Stage	Number of cells analysed	Number (%) of cells with		
		normal disjunction	equational separation	abnormal separation ^a
Anaphase I/ telophase I	112	14 (12.5)	30 (26.8)	68 (60.7)
Anaphase II/ telophase II	160	18 (11.2)	44 (27.5) ^b	98 (61.3)

^a Included abnormalities such as inclusion of two or more alien chromosomes in one and the same pole as well as the lagging ones

^b Possessing a large and a small chromosome in one nucleus and the other two in each of the two other nuclei

In order to estimate the number of large and small alien chromosomes that were included in each of the telophase II nuclei, the composition of 640 telophase II nuclei were scored and the results are given in Table 2. A notable feature was that, despite a deletion, the small alien chromosome was included in telophase II nuclei as frequently as the normal (large) chromosome. In half of the nuclei (50.3%), a single alien chromosome was present whereas in 12.8% of the cases both were included in a

single nucleus. In very few cases either two small (0.6%) or two large (0.3%) chromosomes were present per nucleus (Table 2).

Table 2 Number and type of alien chromosomes included in each of the telophase II nuclei of microsporogenesis

Number and types of chromosomes included	Number of nuclei	% of the total number of nuclei	% of nuclei with alien chromosomes
None	230	35.9	-
1 Large	162	25.3	39.5
1 Small	160	25.0	39.0
1 Large + 1 Small	82	12.8	20.0
2 Small	4	0.6	1.0
2 Large	2	0.3	0.5

Female transmission of alien chromosomes

Unlike microsporogenesis, the cytological distribution of the alien chromosomes during megasporogenesis could not be determined because of technical difficulties for analysing meiosis in the ovules. However, it was possible to determine the frequencies of female transmission of both types of alien chromosomes from BC₂ parent to BC₃ progeny through RFLP and GISH analyses. From the RFLP analysis of BC₃ progeny consisting of 96 individuals, 70 plants (73.0%) did not possess any of the alien chromosomes (Table 3). Through GISH analysis it was established that, in contrast with the scores in microsporogenesis, among the 19 individuals (19.7%) that possessed single alien additions, 18 had the alien large (normal) chromosome, representing 69.2% of the total transmission, and only one possessed the small chromosome, representing 3.8% of the total transmission. Besides single additions, there were seven individuals (7.3%), that had disomic additions

Table 3 Female transmission of alien chromosomes from BC₂ to BC₃ progenies estimated in a BC₃ population of 96 plants through RFLP and GISH analyses

Type of analysis	Number of plants (%)			
	without additions	with additions	with single additions	with two additions
RFLP	70 (73.0)	26 (27.0)	-	-
GISH	70 (73.0) ^a	26 (27.0)	19 (19.7)	7 (7.3) ^b

^a Based on analysis of 15 randomly chosen plants

^b All consisted of one large and a small alien chromosomes

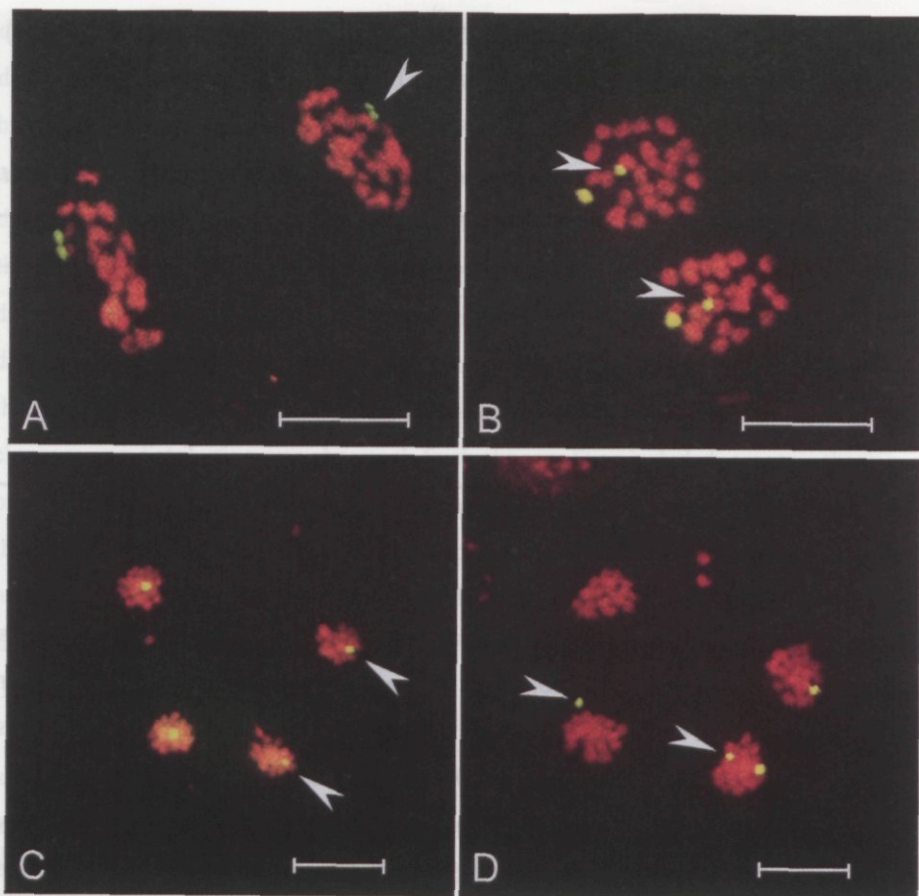


Fig. 2 Disjunction and division of alien chromosomes during microsporogenesis in BC₂ clone 2101-1. **A-D** The alien tomato chromosomes fluoresce yellow due to FITC labelling of tomato genomic DNA whereas potato chromosomes fluoresce orange-red due to propidium iodide counterstain. Scale bars correspond to 10 μ m. **A** Telophase I stage showing a normal disjunction of large and small (arrow heads) alien chromosomes to two poles. **B** Prophase II stage showing equational division of both large and small chromosomes that had occurred during anaphase I. Note: a large and small chromosomes are present at each pole. **C** Normal distribution of four chromatids of alien chromosomes to four poles as a result of normal distribution during the first division followed by normal disjunction during anaphase II. **D** Telophase II stage showing abnormal distribution of the four chromatids of alien chromosomes. One large and a small chromosome are included in one nucleus whereas the others are in different nuclei which leaves one devoid of any alien chromosome

which represented 23.9% of the total transmission. All these disomics consisted of one large and a small chromosome.

All plants in which RFLP analysis detected the alien chromosome, GISH also confirmed the presence of either one or two alien chromosomes. In addition, 15 randomly chosen individuals of that BC₃ population in which the presence of the alien chromosome was not detected through RFLP were also subjected to GISH analysis. Consistently, there was no alien chromosome detected in any of them. The strict correspondence of the results obtained through both techniques (GISH and RFLP) proved that no indications for chimerism were found within those genotypes. In addition, from the observations through GISH analyses there was no evidence for the occurrence of homoeologous recombination or translocations.

Discussion

Identification of the disomic addition

This study demonstrates that despite the smallness of the tomato chromosomes, as well as the high ploidy level of the potato clone investigated ($2n = 4x = 48 + 2$), it is possible to identify the alien chromosomes and pinpoint chromosome aberrations, a deletion in the present case, in BC₂ and BC₃ progenies of potato (+) tomato somatic hybrids. Although the alien chromosomes in these cases can also be unequivocally identified through RFLP analysis alone, it would be very difficult, if not impossible, to determine the number of copies of the alien addition in a plant through genetic analysis. Furthermore, without cytological observations through GISH and FISH analyses, the detection of the interstitial deletion in one of the homologues would have been impossible. Thus, a combination of RFLP analysis together with *in situ* DNA hybridisation techniques enables the use of tomato, with a relatively small nuclear genome, $2C = 2.0$ pg (Bennett and Leitch 1995), for critical molecular cytogenetic analysis in this type of material. The favourable morphological attributes of the pachytene chromosomes of tomato have been very well exploited in the past in traditional cytogenetics (Khush and Rick 1968). The same attributes are equally useful in molecular cytogenetics because the morphological integrity of the pachytene chromosomes is well preserved even after GISH and FISH procedures. In view of this, it should be possible to define or to identify even small chromosomal aberrations, if any, when they occur in an alien background.

Occurrence and meiotic behaviour of the disomic addition

The occurrence of disomic additions of alien chromosomes in the backcross progeny is an advantage. This is especially so in situations where male sterility is the limiting factor for self-pollination, as is frequently observed in most of the backcross derivatives of potato (+) tomato fusion hybrids. Moreover, the highly restricted male transmission of the extra chromosomes that has been described in several other species (reviewed by Khush 1973) is expected to be a great hurdle for producing disomic additions from some of the monosomic addition that we have already selected (Garriga-Calderé et al. 1998). In all these cases, there is a general tendency of the alien chromosomes to behave abnormally during meiosis (Jacobsen et al. 1995; Garriga-Calderé et al. 1997, 1998) and they are expected to generate disomic additions through meiotic products in certain frequencies. In view of the considerable frequency (7.3%) of the disomics obtained in the present backcrossing investigation (Table 3) it might be reasonable to expect that it should be possible to obtain disomic additions in other cases as well.

A notable feature of the smaller alien chromosome 10, which had suffered a deletion, was that it did not behave differently from its normal counterpart. Because it is an interstitial deletion in which the telomeres, sub-telomeres and centromere are intact, its normal behaviour can be explained. However, looking to the reduction of its size relative to the normal homologue, it gives the impression that the deletion is a large one. It is well established that the so-called 'centromeric heterochromatin' that flanks the centromere in all chromosomes of tomato is nearly devoid of functional genes (Khush and Rick 1968). If the deletion is confined largely to the heterochromatic segment, as it is the case in the present study, then the chromosome may not suffer any disadvantage in terms of survival and transmission.

Transmission of the alien chromosomes

The rate of female transmission of the alien chromosome 10 from a BC₂ parent in a disomic condition to BC₃ progeny was fairly high (27.0%). Nevertheless, in the previous study the rate of transmission of this particular chromosome in a monosomic condition varied from 10 to 20% in three different populations (Garriga-Calderé et al. 1998). This means that, although there was an increase in the rate of transmission in a

disomic, it was not as much as one should have expected. Because of the potential for normal bivalent formation in a disomic, a more regular disjunction at anaphase I and a proper mitotic division at anaphase II should be the norm. In view of this, a much higher rate of female transmission of the alien chromosome was expected in the disomic addition. The lower frequency of transmission observed in this study should be attributed to the abnormal meiotic behaviour of the alien chromosome. Abnormalities such as precocious separation of the bivalent at metaphase I and premature division of the half-bivalent(s) at meta/ anaphase I stages lead to lagging of chromatids and their failure to be included in the meiotic products. However, in a previous study where alien chromosome transmission through the female parent was estimated from BC₁ to BC₂ progenies, it was observed that disomic condition for two of the alien chromosomes viz., 2 and 6, resulted in a much higher rate of transmission, 92 and 88% respectively (Garriga-Calderé et al. 1998). Probably, differences in genotypic background and/or the differences among the alien chromosomes might play an additional role in the rate of transmission. Besides genotypic background, the size of the chromosome (Einset 1943) and the presence of a deletion or any other modification can also affect its rate of transmission. Most importantly, the precocious division of the centromeres of the univalents can greatly affect the proper distribution of two chromatids to the poles during meiosis. In view of the low rate of transmission due to meiotic abnormalities in the present disomic addition studied, it remains to be seen whether it is worthwhile to look for similar disomic additions for other chromosomes.

Considering only those nuclei that possessed alien chromosomes, the number of telophase II nuclei which had either a large or a small chromatid were practically the same, 39.5% and 39.0% respectively. In contrast, the frequency of female transmission from BC₂ to BC₃ progenies of these two alien chromosomes (large and small) appeared to be very different. When considering only those BC₃ progenies that possessed alien chromosome additions, 69.2% possessed the large one and only 3.8% had the small one. The exact causes of such a difference are not known but they might arise due to gametic and/or zygotic selection. Despite the differences in the frequencies of female transmission of the large and the small chromosomes, there seemed to be very little differences between the frequencies of alien chromosomes (whether large or

small) in the telophase II nuclei of male meiosis (78.5%) and in the female transmission of the large and the small chromosomes (69.2% + 3.8% = 73.0%). Furthermore, the frequency of telophase II nuclei with both large and small chromosomes (20.0%) was nearly the same as the BC₃ progenies that possessed both chromosomes (23.9%).

All the seven disomics recovered in the present study proved to possess one large and a small chromosome although other possibilities such as two large or two small chromosome additions were also expected to occur. Looking to the types of meiotic products during microsporogenesis, it appears that the frequencies of the later mentioned possibilities are also very low (Table 2). The almost exclusive occurrence of both large and a small homologue in one and the same telophase II nucleus demonstrates that not only two chromatids of a single alien chromosome, or half bivalent are responsible for the occurrence of a disomic (De Jong et al. 1995) but also that two different chromatids of a pair of homologues are by far most likely to be involved in the origin of a disomic. Recovery of alien disomic additions that are completely homozygous (e.g. for the deletion in the present study) can be most useful for physical mapping of the chromosome.

5

Prospects for introgressing tomato chromosomes into the potato genome: an assessment through GISH analysis

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Submitted for publication

Abstract

With a view to assess the possibility of homoeologous pairing and crossing-over between the chromosomes of potato (*Solanum tuberosum*) and tomato (*Lycopersicon esculentum*), a somatic fusion hybrid and two potato genotypes with monosomic alien tomato addition were investigated through genomic *in situ* hybridisation (GISH). The somatic fusion hybrid, C31-17-51, was near hexaploid ($2n = 6x - 4 = 68$) possessing 46 potato chromosomes + 20 tomato chromosomes + 2 translocated chromosomes. The two potato genotypes with alien addition were near tetraploids ($2n = 4x + 1 = 49$) and consisted of monosomic alien additions for tomato chromosome 1 in genotype 2103-1, and tomato chromosome 8 in genotype 2301-2. In the fusion hybrid the tomato pachytene chromosome identification revealed that the chromosomes 1, 2, 5, 6, 7, 10 and 12 were in a diploid condition whereas among those that were in a haploid condition, three could be identified viz., 4, 9 and 11. The remaining three chromosomes could not be cytologically identified. Although the chromosomes with translocated segments could not be identified at the pachytene stage due to technical difficulties, there was clear evidence for the presence of a reciprocal translocation observed at diakinesis and metaphase I stages. Because of autosyndetic pairing of the translocated segments, it gave a false impression as if there was a high frequency (86.0%) of allosyndetic pairing. In contrast to the fusion hybrid, the two potato genotypes with alien monosomic addition showed a very low frequency of allosyndetic pairing, namely 1.1 and 1.3% respectively for the monosomic additions 1 and 8. In the genotype 2301-2, monosomic addition for tomato chromosome 8, crossing-over between the homoeologous chromosomes was estimated to occur in 0.8% of the meiotic cells investigated. Despite this low frequency of homoeologous pairing and crossing-over, there is a possibility for introgressing tomato chromosomal DNA into the potato genome through intergenomic recombination.

Introduction

There are arguments in literature that the genera, *Solanum* and *Lycopersicon*, both belonging to the Solanaceae family, have evolved independently for more than 100 million years (Hawkes and Smith 1965). Despite such a long period of separate evolution, the genomes of these genera are highly conserved. For example, the pachytene chromosome morphology of potato and tomato is remarkably similar (Ramanna and Wagenvoort 1976) and, so also, the genetic linkage maps of these taxa, based on restriction fragment length polymorphism (RFLP) markers, are homosequential (Tanksley et al. 1992). Considering these similarities, the questions arise whether (i) there is affinity for homoeologous pairing of chromosomes in the hybrids of these two genera, (ii) crossing-over between homoeologous chromosomes occurs during meiosis, and (iii) there are prospects for introgression of chromosomes (or parts) from one genus into the other.

In the sexual hybrids between *Lycopersicon esculentum* x *Solanum lycopersicoides* and their backcross derivatives, the occurrence of homoeologous pairing and crossing-over has been demonstrated (Menzel et al. 1962; De Verna et al. 1987; Chetelat et al. 1989; 1997). Phylogenetically, genomes of the parents involved in intergeneric hybrids between the non-tuberous taxa are expected to be much more closely related than those between tuberous and non-tuberous taxa such as potato and tomato. Although sexual hybridisation of potato and tomato is not possible, somatic fusion hybrids have been successfully produced (Melchers et al. 1978; Shepard et al. 1983; Jacobsen et al. 1992; Schoenmakers et al. 1992). Using these somatic fusion hybrids, the aspect of homoeologous pairing between the chromosomes of potato and tomato has been analysed at the level of synaptonemal complexes and metaphase I stages during microsporogenesis (De Jong et al. 1993; Wolters et al. 1994). From these studies it was inferred that there is a considerable amount of homoeologous pairing between the chromosomes of potato and tomato. The important drawbacks of these analyses were that the presence of interchanges between the potato and tomato chromosomes that frequently occur in these somatic fusion hybrids (Shepard et al. 1983; Wolters et al. 1994; Garriga-Calderé et al. 1997) could give a false impression

of allosyndetic pairing between the chromosomes of the two genomes, and furthermore, that there was no direct evidence for the involvement of the tomato chromosomes in each of the paired multivalent configurations that were observed in those studies.

It is important to resolve the question of homoeologous chromosome pairing and crossing-over in potato (+) tomato fusion hybrids and their backcross derivatives for two reasons. Firstly, without homoeologous pairing and recombination, introgression of desirable segments of alien chromosomes would be difficult. Secondly, if there is a high rate of recombination between the chromosomes of the two genomes, it will be very difficult, if not impossible, to establish monosomic alien additions through backcrosses, because recombination during meiosis can disrupt the integrity of the alien chromosome. With the aim of establishing a complete series of tomato chromosome additions into the potato genome, seven out of the twelve possible monosomic alien additions have already been identified (Garriga-Calderé et al. 1998). In order to assess the consequences of adding tomato chromosomes into the potato genome, more critical information regarding homoeologous chromosome pairing and crossing-over in the somatic fusion hybrid and the backcross derivatives was essential. These aspects were investigated through GISH analysis in a somatic fusion hybrid and two backcross derivatives, viz., monosomic additions for chromosome 1 and 8.

Materials and methods

Plant material

A near hexaploid ($2n = 6x - 4 = 68$) potato (+) tomato fusion hybrid and two near tetraploid potato genotypes with monosomic alien tomato addition were used. The fusion hybrid, C31-17-51, possessed 46 potato chromosomes plus 20 tomato chromosomes plus two chromosomes with translocated segments (Garriga-Calderé et al. 1997). The two near tetraploid ($2n = 4x = 48 + 1$) potato genotypes with alien monosomic tomato addition consisted of an addition for chromosome 1 (clone 2103-1) and an addition for chromosome 8 (clone 2301-2) (Garriga-Calderé et al. 1998).

GISH analysis

For meiotic studies, young anthers with suitable division stages were fixed in ethanol:acetic acid (3:1) for about 30 min at room temperature. Chromosome spreads on a grease-free slide were made according to the technique of Pijnaker and Ferwerda (1984). Total genomic DNA of tomato was labelled with digoxigenin-11-dUTP following a standard nick-translation protocol (Boehringer Mannheim). The protocol to perform genomic *in situ* hybridisation was similar to that described by Schwarzacher and Heslop-Harrison (1994). The hybridisation mixture, conditions, and stringency washings, were the same as those previously described (Garriga-Calderé et al. 1997). The detection of digoxigenin with anti-dig-FITC (fluorescein isothiocyanate), counterstaining and photographic procedures were those of Garriga-Calderé et al. (chapter 4). The tomato chromosomes were identified at pachytene stages based on their morphology according to Ramanna and Prakken (1967).

Results

For the analysis of chromosome pairing configurations, early to late prophase I, viz., pachytene and diakinesis as well as metaphase I stages of microsporogenesis, were investigated. Owing to the high ploidy levels of the plants used chromosome spreading was difficult. Nevertheless, because of the differential green-FITC-fluorescence of the tomato chromosomes after GISH, pachytene stages were informative enough to allow their individual identification. It should be stressed that pachytene chromosome morphology in GISH preparations corresponded completely with the details observed in traditionally stained preparations. Chromosome pairing configurations, e.g. bivalents at pachytene stages, were studied in all three genotypes. However, the occurrence of chiasmata formation at diakinesis and metaphase I stages, could only be estimated in the somatic fusion hybrid C31-17-51, and in the monosomic addition for chromosome 1, clone 2301-2.

Alien tomato chromosome identification and pairing in the somatic hybrid

A previous study based on RFLP analysis indicated that all 12 individual tomato chromosomes were present in the somatic hybrid C31-17-51. However, GISH analysis

on somatic cells detected only 20 tomato chromosomes and two translocated chromosomes in addition to the 46 potato chromosomes (Garriga-Calderé et al. 1997). This obviously led to the conclusion that some of the tomato chromosomes were present in a disomic condition, as expected, whereas some others were present in a monosomic condition. This was indeed confirmed in the present study where both bivalents and univalents were observed at different stages of meiosis. These structures could be distinguished at pachytene stage because the bivalents were clearly thicker (Fig. 1A, *blue arrow heads*) than the univalents (Fig. 1A, *white arrow heads*). From an analysis of more than ten cells, it was possible to unequivocally identify all the chromosomes that formed bivalents as well as most of those that remained as univalents. In all, there were seven pairs of tomato chromosomes that formed bivalents regularly (*viz.*, chromosomes 1, 2, 5, 6, 7, 10 and 12) and among those that remained as univalents, three were identified (4, 9 and 11). Of the remaining three univalents two could not be identified unequivocally due to partial pairing (Fig. 1A *arrow*), and in the other the morphology was mostly distorted. Except for two tomato chromosomes, 3 and 8, all others could be accounted for either as forming bivalents or univalents. Because of technical difficulties it was not possible to identify the two chromosomes that were involved in translocations at pachytene stage which were previously detected in the somatic cells of this somatic fusion hybrid (Garriga-Calderé et al. 1997). At the later stage of diakinesis, however, there was clear evidence for the presence of translocated segments because of the formation of quadrivalent configurations. These configurations included either a chain or a ring of four, obviously involving two chromosomes with translocated segments and the normal homologues, one chromosome of potato and one of tomato (Figs. 1B and 1C). Besides these, two bivalents, each involving one of the translocated segments either with a potato or a tomato chromosomes were frequently observed at metaphase I (Fig. 1D). Based on these results, it was concluded that these pairing configurations were the result of a reciprocal translocation which had occurred in the somatic fusion hybrid.

An estimate of 93 pollen mother cells in this somatic fusion hybrid at either diakinesis or metaphase I stages showed that in 86.0% of the cases there was at least one association between a translocated chromosome and a potato or tomato chromosomes (Table 1).

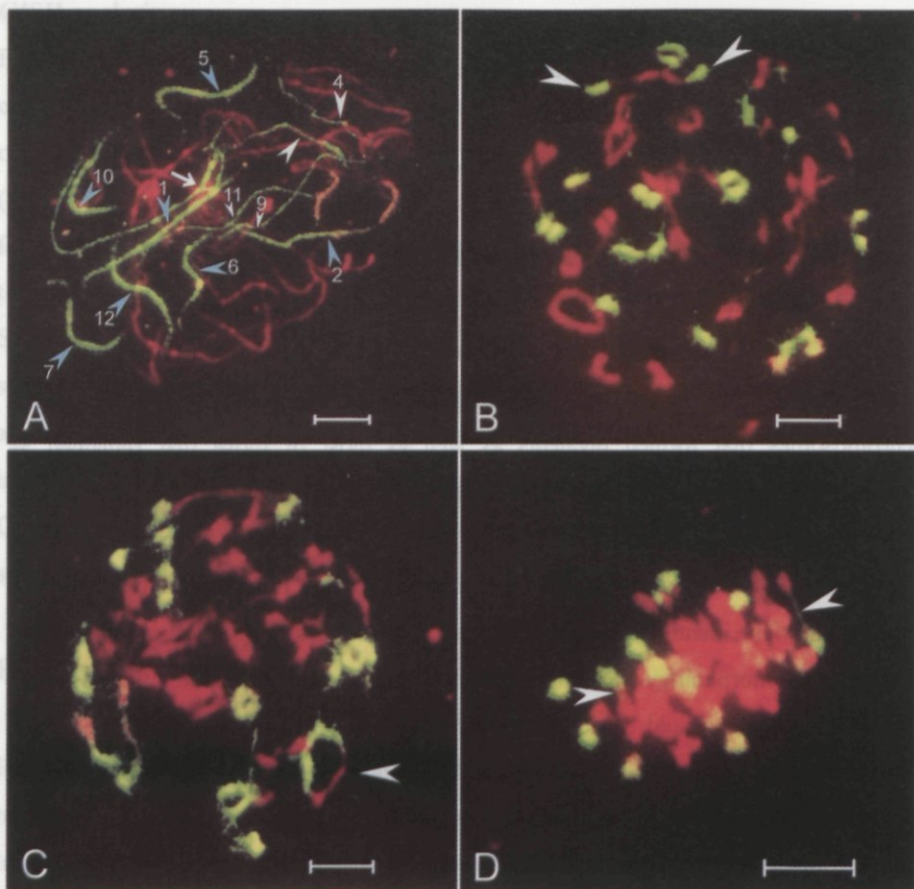
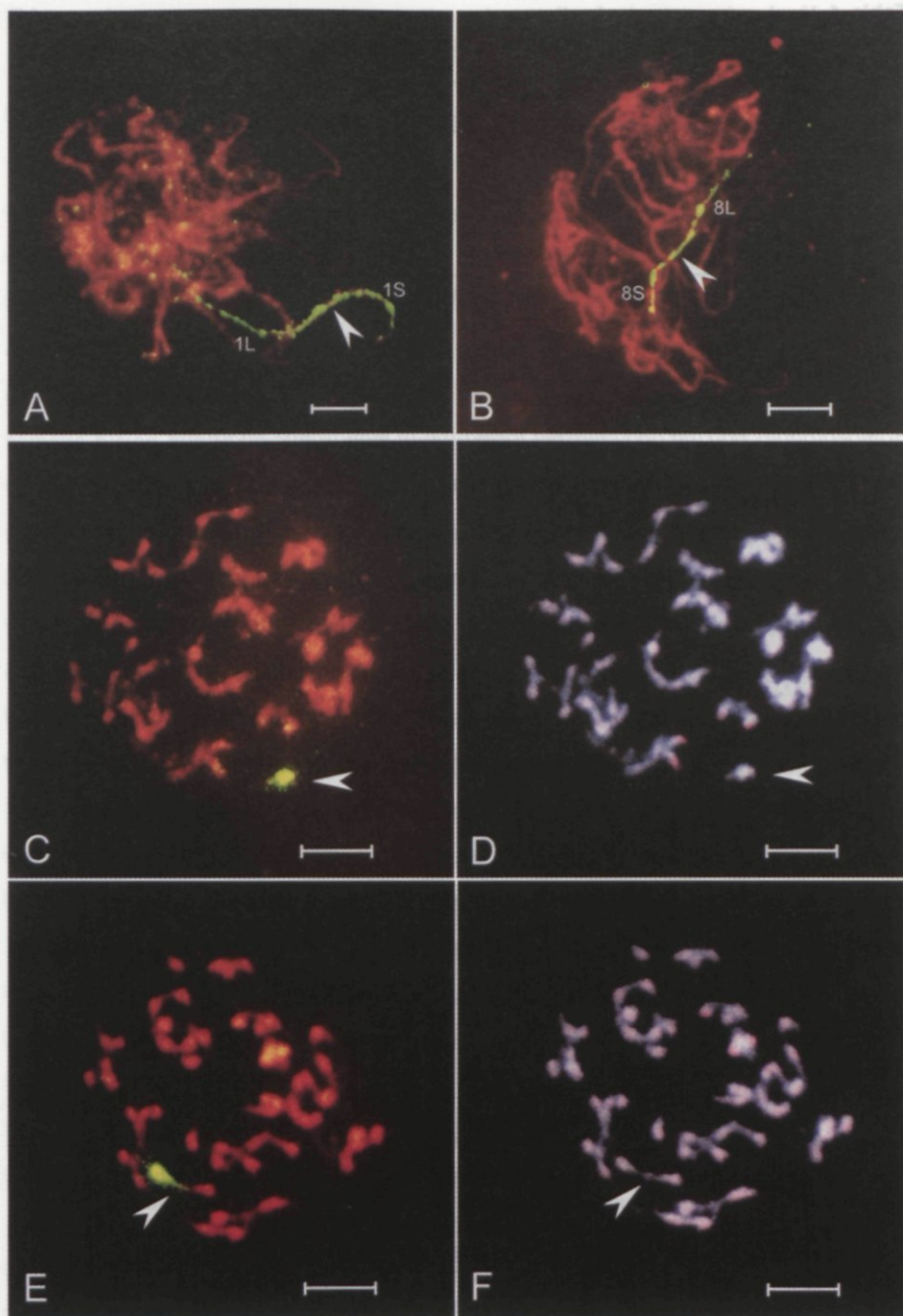


Fig. 1 Chromosome constitution and meiotic behaviour of alien tomato chromosomes in the somatic fusion hybrid C31-17-51. The alien tomato chromosomes fluoresce green due to fluorescein isothiocyanate (FITC) labelling of tomato genomic DNA whereas potato chromosomes fluoresce red due to propidium iodide counterstain. Scale bar represent 10 μm . **A** The pachytene stage in which the alien tomato chromosomes were identified based on their morphology. Bivalents and univalents are indicated with blue and white arrow heads, respectively. Arrow heads in the chromosomes identified indicate the position of the centromeres. The arrow shows a partial bivalent. **B** A diakinesis stage showing the two translocated chromosomes paired with their respective homologues, one of potato and one of tomato in a multivalent chain configuration (arrow heads). **C** A diakinesis stage showing a multivalent, ring of four (arrow head), involving the two translocated chromosomes and two normal chromosomes, one of potato and one of tomato. Notice that this configuration can only be formed when the translocations are reciprocal. **D** Metaphase I stage showing the two translocated chromosomes forming bivalents (arrow heads). Because of the translocations this is autosyndetic pairing



chromosomes is due to their association with the paired homologous C. Distinct tags in the
 quantitative 2D11.5 nonoverlapping addition for chromosome 8, showing the sister chromosome as a
 univalent (arrow head). D. The corresponding cell to C counterstained with 4',6-diamino-2-
 phenylindole (DAPI) showing sister chromatid exchange (arrow head) which is a clear indication of
 recombination. The corresponding cell counterstained with DAPI showing sister chromatid exchange
 the conclusion that a relative relationship exists with the

Table 1 Number (percentage) of pollen mother cells in which homoeologous associations were observed in a somatic fusion hybrid and in two potato genotypes with monosomic additions through GISH

Genotype	Stage analysed	Nr. of cells analysed	Nr. of cells (%) showing homoeologous associations
C31-17-51 (Somatic hybrid)	Metaphase I	93	80 (86.0) ^a
2103-1 (Addition chr. 1)	Pachytene	230	3 (1.3)
2301-2 (Addition chr. 8)	Pachytene	267	3 (1.1)
“ “	Diakinesis	816	7 (0.8)

^a at least one instance of homoeologous association was observed due to translocated chromosomes. Therefore, it was autosyndetic pairing

Homoeologous pairing and chiasma formation in monosomic additions

In the two potato genotypes with monosomic alien tomato additions, homoeologous chromosome pairing at pachytene stage was estimated in more than 200 cells (Table 1). The criteria for pairing between the homoeologous chromosomes were either the presence of a completely paired bivalent in which both red and green homoeologues were evident (Fig. 2B), or even the homoeologous parts were associated (Fig. 2A). In all, the frequencies of cells showing allosyndetic pairing were very low (Table 1). The estimates were 1.3% for the alien monosomic addition for chromosome 1 (Fig. 2A) and 1.1% for the alien monosomic addition for chromosome 8 (Fig. 2B). The low frequency was also evident at the latter stage of diakinesis, where the alien tomato chromosome 8, in clone 2301-2, was observed as a univalent in most cases (Figs. 2C and D). It was only in 0.8% of the cases (Table 1) that rod bivalent associations (Figs. 2E and F), and occasionally multivalent chain formations involving the alien tomato chromosome with two or more potato chromosomes were observed (data not shown).

Fig. 2 Pairing behaviour of two alien tomato monosomic addition genotypes. The alien tomato chromosomes fluoresce green due to fluorescein isothiocyanate (FITC) labelling of tomato genomic DNA whereas potato chromosomes fluoresce red due to propidium iodide counterstain. Scale bars correspond to 10 μ m. **A-B** Pachytene stages showing allosyndetic pairing in the genotypes 2301-1 and 2301-2, monosomic alien tomato additions for chromosome 1 and 8, respectively. *Arrow heads* show the positions of the centromeres. Notice that the well preserved morphology of the alien tomato chromosomes is due to their association with the potato homologues. **C** Diakinesis stage in the genotype 2301-2, monosomic addition for chromosome 8, showing the alien chromosome as a univalent (*arrow head*). **D** The corresponding cell to **C** counterstained with 4',6-diamino-2-phenylindole (DAPI). **E** Diakinesis stage in the genotype 2301-2, monosomic addition for chromosome 8, showing allosyndetic association (*arrow head*) which is a clear indication of chiasma formation. **F** The corresponding cell to **E** counterstained with DAPI

Discussion

This investigation proves that the translocated chromosomes detected in the somatic cells of the somatic fusion hybrid C31-17-51 (Garriga-Calderé et al. 1997), are indeed a pair representing a reciprocal translocation between potato and tomato chromosomes. Such interchanges between the chromosomes of alien genomes have also been encountered in somatic fusion hybrids of other distantly related taxa such as *S. nigrum* (+) *S. tuberosum* (K. Horsman and E. Jacobsen, unpublished). A critical confirmation was required because such interchanges occur exceptionally in the somatic fusion hybrid itself, i.e. without the intervention of meiosis. This means that the event is not associated either with homoeologous meiotic pairing or with recombination. The reasons for their occurrence are unknown, but it appears that chromosomes of potato genotypes are predisposed to suffer different types of rearrangements when they pass through a callus phase during *in vitro* regeneration (Creissen and Karp 1985). In this connection, translocations between the potato and tomato genomes in potato (+) tomato somatic fusion hybrids were already anticipated by Shepard et al. (1983) who observed octavalent formation in pollen mother cells. Moreover, their occurrence has also been demonstrated by Wolters et al. (1994) and Garriga-Calderé et al. (1997). When accurately identified, such rearrangements can be most useful for physical mapping of chromosomes as has been demonstrated in wheat (Badaeva et al. 1995).

Because of the high ploidy level of the somatic fusion hybrid it was quite difficult to obtain cytological preparations with well-spread chromosomes. Nevertheless, informative pollen mother cells could be obtained in considerable numbers as is evident from Figure 1A. Because the pachytene chromosomes of tomato are amenable for identification, it was possible to identify the chromosomes that did not suffer aberrations. Even a tentative identification of the aberrant chromosomes (e.g. chromosomes 3 and 8 in C31-17-51 in the present study) can be most useful for the further development of this type of material for genetic studies.

Previous meiotic studies on chromosome pairing in potato (+) tomato somatic fusion hybrids have indicated a considerable amount of allosyndetic pairing, leading to the conclusion that a relatively high level of homoeologous recombination between the

potato and tomato genomes could be expected (De Jong et al. 1993, 1995; Wolters et al. 1994). However, the limitations of these studies were (i) that the possibility of the presence of chromosome interchanges were not taken into account, and (ii) that actual chromosome association between the alien chromosomes could not be directly established in the meiotic configurations. In the present investigation, the occurrence of allosyndetic pairing has indeed been observed but not to the extent that was shown to occur in the previous studies. The high frequency of associations between the alien chromosomes observed in the somatic fusion hybrid C31-17-51, (86.0%, Table 1), could have been mistaken for allosyndetic pairing if the presence of reciprocal translocations had not been established. In this case, however, it was solely due to autosyndetic pairing between the translocated segments and their normal homologues of potato. Moreover, GISH is a powerful tool to demonstrate that alien chromosomes are indeed involved in the multivalent association and that this association is due to a reciprocal translocation. In fact, an exclusive occurrence of autosyndetic pairing in other potato (+) tomato somatic fusion hybrids and BC progenies that possessed intact chromosomes, viz., without visible translocations, has been repeatedly observed in previous studies (Garriga-Calderé et al. 1997, 1998). Therefore, it was essential to elucidate whether allosyndetic pairing and homoeologous recombination occurs at all, and if it does, in what frequency. One critical method of proving allosyndetic pairing and homoeologous crossing-over was through the analysis of pairing in genotypes such as 2103-1 and 2301-2, the monosomic alien tomato additions for chromosomes 1 and 8, respectively. A very low frequency of allosyndetic pairing was observed in both cases at pachytene stage (Table 1). In addition, there were clear indications for the occurrence of chiasmata between the chromosomes from both genomes as was evident from bivalent formation at diakinesis (Figs. 2E and D), which also occurred at a low frequency (Table 1). In spite of this, there is a possibility for introgressing chromosomal DNA through homoeologous recombination from tomato into potato. The restricted frequency of homoeologous pairing and subsequent recombination between the chromosomes of potato and tomato, in combination with the considerable stability of the alien tomato chromosomes in this material, makes it highly attractive for genetic studies. However, in view of the very low frequency of crossing-over

between the tomato and potato chromosomes, selection of genotypes with recombinant chromosomes can be quite laborious.

6

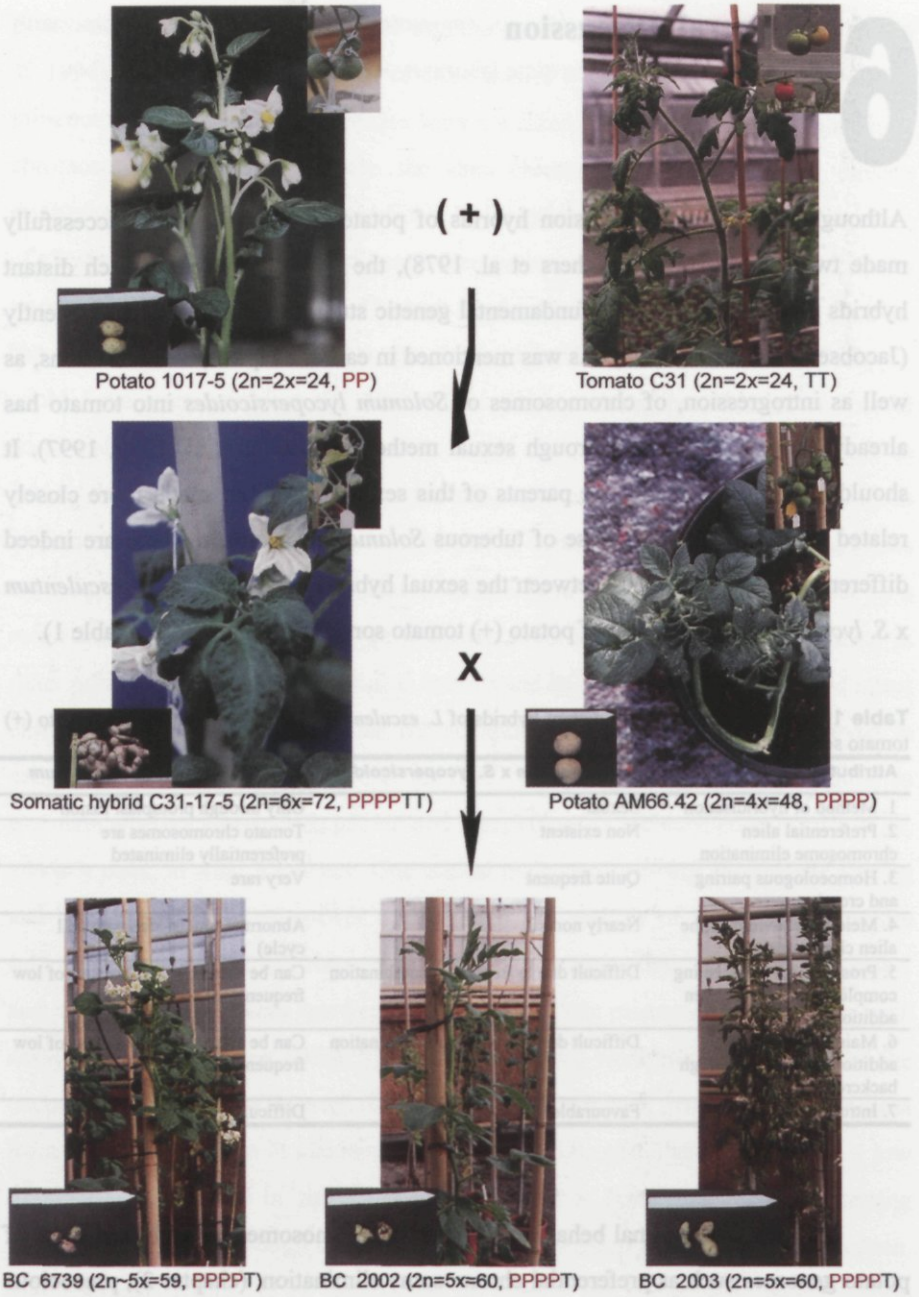
General Discussion

Although the first somatic fusion hybrids of potato and tomato were successfully made two decades ago (Melchers et al. 1978), the prospects of using such distant hybrids for introgression and fundamental genetic studies has emerged only recently (Jacobsen et al. 1994, 1995). As was mentioned in earlier chapters, alien additions, as well as introgression, of chromosomes of *Solanum lycopersicoides* into tomato has already been accomplished through sexual methods (Chetelat et al. 1989, 1997). It should be emphasised that the parents of this sexual hybrid are much more closely related to each other than those of tuberous *Solanum* and tomato. There are indeed differences in the behaviour between the sexual hybrids of *Lycopersicon. esculentum* x *S. lycopersicoides* and that of potato (+) tomato somatic fusion hybrids (Table 1).

Table 1 Differences between the distant hybrids of *L. esculentum* x *S. lycopersicoides* and potato (+) tomato somatic fusion hybrids.

Attributes	<i>L. esculentum</i> x <i>S. lycopersicoides</i>	<i>S. tuberosum</i> (+) <i>L. esculentum</i>
1. Method of hybridisation	Sexual	Only through protoplast fusion
2. Preferential alien chromosome elimination	Non existent	Tomato chromosomes are preferentially eliminated
3. Homoeologous pairing and crossing-over	Quite frequent	Very rare
4. Meiotic behaviour of the alien chromosome	Nearly normal	Abnormal (out of step with cell cycle)
5. Prospects for establishing complete monosomic alien additions	Difficult due to frequent recombination	Can be accomplished because of low frequency of recombination
6. Maintenance of alien addition genotypes through backcrosses	Difficult due to frequent recombination	Can be accomplished because of low frequency of recombination
7. Introgression mapping	Favourable	Difficult

In spite of abnormal behaviour of tomato chromosomes in the background of potato genomes such as preferential chromosome elimination, (chapter 2), precocious disjunction and division, (chapters 2, 3 and 4), and high degree of sterility, it is possible to establish genotypes with alien tomato addition. Persistence of alien chromosomes in the somatic fusion cells as well as their transmission to the BC₂ and BC₃ progenies in fairly high frequencies indicates that the alien addition genotypes can



be further used in introgression and other genetic studies. Because seven out of the possible 12 types of monosomic addition genotypes have already been identified in this study, with some additional effort, there is a good prospect for completing the entire series within the near future.

One drawback of the addition genotypes is that the alien tomato chromosomes have been added into a tetraploid potato background. Although some morphological traits in the potato (+) tomato somatic fusion hybrids are intermediate, e.g. flower and leaf morphology (see Fig. 1), tomato smell etc., morphological tomato traits disappear in the backcross progenies. Regardless of the type of additional tomato chromosomes present in a genotype, all backcross progenies exhibit the potato phenotype (unpublished results). This is not surprising in view of the overwhelming predominance of the potato genomes. One way of expressing phenotypic tomato traits in a potato background is to completely substitute each of the tomato chromosomes individually. At present, it is impractical to accomplish such a feat in tetraploid potato in view of the polysomic condition, the problem of inbreeding depression and high degree of sterility. An alternative could be to generate diploid material from the present tetraploid condition (Hermsen and Verdenius 1973) and select for monosomic or disomic alien additions and preferably for substitutions at the diploid level. In this way, morphological tomato traits could be located on particular chromosomes. Although not impossible, the problems of loss of vigour and high degree of sterility that are expected to occur in dihaploids, impose serious limitations to create alien tomato monosomic and disomic addition genotypes at the diploid level. The availability of monosomic alien addition genotypes in a tetraploid background does not, however, limit their usefulness for introgression, molecular cytogenetic studies and genetic mapping. In bread wheat which is hexaploid, for example, as a first step in introgression, alien addition lines are produced and characterised (Jiang et al. 1994). From such lines, genotypes with homoeologous recombinations (or translocations) are selected and used further for introgression of specific traits. Fortunately, in wheat

Fig. 1 Some morphological features of the initial protoplast fusion parents of the somatic hybrids, tomato (*L. esculentum*) and potato (*S. tuberosum*), somatic fusion hybrid C31-17-24 and backcross progenies. The plastic label besides the tubers measures 20 cm.

there are genetic approaches such as the use of the *ph* genes that can induce homoeologous recombination (Chen et al. 1994). In the absence of such genetic methods, in potato (+) tomato somatic fusion hybrids, the only means to obtain genotypes with either homoeologous recombination or translocations between the chromosomes of the two genomes is to utilise those that are rarely found in potato (+) tomato somatic fusion hybrids (chapters 2 and 5) and their backcross derivatives (chapters 2).

The already identified genotypes with single alien tomato chromosome additions (chapter 3) together with a deletion (chapter 4) and a reciprocal translocation (chapter 5) can be most useful for molecular cytogenetic studies. Because of the considerable phylogenetic distance between *Solanum* and *Lycopersicon*, it should be possible to identify and molecularly isolate species specific repetitive DNA sequences. Such DNA sequences could be used as probes to identify DNA fragments from total genomic digests of each of the individual monosomic alien addition genotypes. This would obviously open the prospects of establishing tomato chromosome specific DNA libraries as has been demonstrated for alien maize monosomic additions into the oat genome (Ananiev et al. 1996).

Identification of a deletion for the heterochromatic part of chromosome 10 of tomato (chapter 4) is an example of how easily identifiable pachytene chromosomes of tomato can be used for mapping (cytologically) a deletion. Genotypes with such deletions can be useful for physical mapping of tomato chromosomes, comparable to that demonstrated in wheat (Endo and Gill 1996). Another interesting event was the reciprocal translocation that was detected in the somatic fusion hybrid C31-17-15, (chapters 2 and 5). The exact cause of such chromosomal exchanges of alien segments between the chromosomes of different genomes is not clear. But they have been reported to occur frequently in other distant hybrids such as those of *Nicotiana* species (reviewed by Smith 1968) and of cereals (Jiang and Gill 1994). Genotypes consisting of a single chromosome with an exchange can be profitably used for physical mapping as has been demonstrated in wheat (Werner et al 1992). Nevertheless, plants possessing such translocated chromosomes in a single condition identified in the present study, were either weak or did not flower (unpublished results). The reason for

this is unknown but similar observations have also been reported in the case of oat genotypes carrying oat-maize chromosome interchanges (Riera-Lizarazu et al. 1996).

Plants with small genomes such as *Arabidopsis* and rice, $2C=0.3$ pg. and $2C=1.0$ pg. respectively (Bennett and Leitch 1995), are favourable for molecular cytogenetic studies as compared to those with large chromosomes, e.g. wheat, $2C=36.1$ pg., maize, $2C=5.6$ pg., or *Alstroemeria*, $2C=36.5-78.9$ pg. (Buitendijk et al. 1997). From the point of view of genome size, tomato with a $2C$ DNA value of 2.0 pg. occupies an intermediate position. Apart from this, the genome of tomato has been much more extensively explored through traditional cytogenetic methods than any other plant species, except maize (Khush and Rick 1968). Therefore, tomato has been considered as an ideal model system for molecular biological studies. Because of the availability of the considerable knowledge on the chromosome organisation at the classical cytogenetic level (Ramanna and Prakken 1967), and also because of the well developed genetic and molecular linkage maps (Tanksley et al. 1992), tomato is expected to be highly suitable for further investigations. In this context, the establishment of a complete series of monosomic alien addition genotypes will be a significant addition for further development of this model system. The availability of all the individual tomato chromosome additions in a potato background can facilitate the analysis of the molecular organisation of individual chromosomes. For example, the localisation of Ty copia-like retrotransposon sequences on the individual chromosomes of tomato is now possible (Kuipers et al. in preparation). Furthermore, the pachytene chromosomes are well differentiated into the so-called eu- and hetero-chromatic regions and most of the functional genes are confined to the euchromatic regions (Kush and Rick 1968). In order to further elucidate such traditional cytogenetic findings through molecular methods, the genotypes with aberrant alien addition chromosomes such as that for chromosome 10 of tomato (chapter 4) can be highly relevant. Because of the accuracy with which the tomato chromosomes can be identified at pachytene stage, even after GISH and FISH procedures, there is a potential that more of such aberrant forms can be detected and used for molecular genetic analysis.

While comparing syntenic relationships between the RFLP linkage maps of

potato and tomato some inversions and translocations in the chromosomes, 5, 9, 10, 11, and 12 have been postulated (Tanksley et al. 1992). The availability of monosomic alien tomato addition genotypes in a potato background can provide a mean of more direct assessment of such structural differences.

Summary

Transfer of alien chromosomes and genes across intergeneric boundaries can be useful not only for the introgression of desirable characters but also for fundamental genetic studies. The successful demonstration of hybridisation of potato (*Solanum tuberosum*) and tomato (*Lycopersicon esculentum*) through protoplast fusion in 1978, created the potential for introgressing chromosomes and genes from one genus into the other. However, real prospects of adding tomato chromosomes into the potato genome arose only after a potato (+) tomato fusion hybrid was successfully backcrossed to tetraploid potato in 1994. Despite this achievement, the problem of selective alien tomato chromosome elimination from the fusion hybrid and the backcross progenies, as well as the behaviour of the alien tomato chromosomes in the potato background, raised the following questions: (i) in view of the selective elimination of alien tomato chromosomes, is it possible to establish a complete series of alien tomato chromosome additions in a potato background? (ii) do the introgressed alien chromosomes retain their structural integrity? (iii) can all the alien tomato chromosomes be transmitted through the gametes to the sexual progeny and if so, in what frequency? (iv) if there are any structural changes in the introgressed alien chromosomes, can they be identified and characterised? (v) do the potato and tomato chromosomes pair homoeologously and cross-over?

An attempt was made in the present investigation to create the appropriate plant material to answer the five above mentioned questions. In this connection, the chromosome constitution of the somatic fusion hybrids and their backcross derivatives, viz., BC₁, BC₂ and BC₃ progenies were analysed through a combination of restriction fragment length polymorphism (RFLP) using tomato chromosome specific probes, genomic *in situ* hybridisation (GISH) and fluorescence *in situ* hybridisation (FISH) analyses.

In order to produce BC₁ progenies with the full potential for the creation of all the 12 possible monosomic tomato chromosome additions in a tetraploid potato background, three different hexaploid somatic fusion hybrid genotypes, viz., C31-17-5, C31-17-24, and C31-17-51, were used successfully as female parents in crosses with four tetraploid potato clones; cv Katahdin, cv Frieslander, 6704-1 and AM 66.42

(chapter 2). From these crosses, several BC₁ progenies were obtained. Among these, six BC₁ plants, viz., 6739, 2001, 2002, 2003, 2004 and 2005 were analysed through RFLP and GISH analyses. Due to preferential alien tomato chromosome elimination, a variable number of tomato chromosomes (6-11) was present in these genotypes. An important result was that all the 12 different tomato chromosomes were present in these six BC₁ progeny plants, albeit in different genotypes.

By backcrossing three of these BC₁ plants to tetraploid potato, a total of 97 BC₂ plants were obtained and analysed through RFLP and GISH (chapter 3). The number of alien tomato chromosomes in these BC₂ plants varied from 0-6. The average rate of tomato chromosome transmission among different populations ranged from 1.7 to 3.4. Generally, a much higher rate of transmission was observed when the alien tomato chromosome in the BC₁ plant was in a disomic condition. A statistical analysis of the rate of female transmission could not prove an equal probability of chromosome transmission among the individual tomato chromosomes. A notable result of this investigation on the BC₂ plants was that among the 12 possible types of monosomic additions seven, viz., chromosome 1, 2, 4, 6, 8, 10 and 12, were identified. This clearly indicated that, despite the problem of selective elimination of the alien tomato chromosomes, it is possible to select genotypes that retain the alien chromosomes in a stable condition and transmit them through the gametes to the progeny.

Besides monosomic additions, an occasional disomic was also detected through RFLP and GISH analyses (chapter 4). Remarkably, one of the chromosomes of this pair in the BC₂ plant 2101-1, was much smaller than the other. A detailed analysis of microsporogenesis indicated that at pachytene stage the large and small tomato chromosomes paired as homologues, and morphologically it was identified as chromosome 10 of tomato, which was also concurrent with RFLP analysis using chromosome specific probes. Morphologically, a deletion for the heterochromatic part of the long arm of the chromosome 10 of tomato had given rise to the smaller of this homologous pair. A detailed analysis of microsporogenesis indicated that a precocious disjunction and division of the alien pair of chromosomes was the cause of the origin of disomic additions in this material. It was evident from this study that the pachytene chromosomes of tomato can be clearly identified after GISH procedures even in a

polyploid genotype and the deletion could be clearly characterised. In addition, a BC₃ population consisting of 96 plants was created and characterised through RFLP and GISH analyses after backcrossing the disomic addition to a tetraploid potato pollen parent. Therefore, the female transmission of both the normal and the aberrant homologues could be determined.

To gain insight into homoeologous pairing and crossing-over between the potato and tomato chromosomes, a somatic fusion hybrid, C31-17-51, with two translocated chromosomes, and two genotypes, 2103-1 and 2301-2, with monosomic alien tomato additions for chromosome 1 and 8 respectively, were investigated (chapter 5). There was clear evidence for the presence of a reciprocal translocation in the somatic fusion hybrid. As a consequence, the quadrivalent formation involving potato and tomato chromosomes was only due to the autosyndetic pairing. In the absence of any knowledge of this translocation, the high degree of pairing, 86.0%, could have been mistaken for allosyndetic pairing. An analysis of chromosome pairing and chiasmata formation in the monosomic additions indicated a very low frequency of homoeologous pairing, 1.3 and 1.1% for the chromosome additions 1 and 8 respectively. Chiasma formation between homoeologues chromosomes was estimated to occur in 0.8% of the meiotic cells studied in the monosomic alien addition for chromosome 8.

The investigations presented in this thesis demonstrated that, although the frequency of homoeologous pairing is low, there is a prospect for introgressing tomato chromosomal DNA into the potato genome.

Samenvatting

De overdracht van soortvreemde chromosomen en genen tussen verschillende genera is niet alleen nuttig voor introgressie van gewenste eigenschappen maar ook voor fundamenteel genetisch onderzoek. Door de succesvolle hybridisatie van aardappel (*Solanum tuberosum*) en tomaat (*Lycopersicon esculentum*) via protoplastenfusie in 1978 ontstond de mogelijkheid tot introgressie van chromosomen en genen tussen soorten uit verschillende genera. Echt goede mogelijkheden voor de toevoeging van tomatenchromosomen aan het aardappelgenoom ontstonden echter pas nadat een aardappel (+) tomaat hybride met succes was teruggekruist met een tetraploïde aardappel in 1994. Ondanks dit resultaat leidden enerzijds de selectieve eliminatie van de soortvreemde tomatenchromosomen uit de fusie-hybride en de terugkruisingsnakomelingen en anderzijds het gedrag van de tomatenchromosomen in de aardappelachtergrond tot de volgende vragen: (i) is het, in het licht van de selectieve eliminatie van soortvreemde tomatenchromosomen, mogelijk om een volledige serie additielijnen van tomatenchromosomen in een aardappelachtergrond te realiseren? (ii) behouden de soortvreemde chromosomen hun structurele integriteit na introgressie? (iii) kunnen alle soortvreemde tomatenchromosomen via de gameten worden overgedragen naar de seksuele nakomelingschap en zo ja, met welke frequentie? (iv) kunnen eventuele structurele veranderingen in de soortvreemde chromosomen na introgressie worden herkend en gekarakteriseerd? (v) treedt er tussen de aardappel- en tomatenchromosomen homoeologe paring en overkruising op?

In het hier beschreven onderzoek werd een poging gedaan om het juiste plantmateriaal te verkrijgen om bovenstaande vragen te kunnen beantwoorden. Hiertoe werd de chromosomale samenstelling van de somatische fusiehybriden en hun terugkruisingsproducten, te weten BC₁, BC₂ en BC₃ nakomelingen, geanalyseerd door middel van een combinatie van restrictie fragment lengte polymorfisme (RFLP) analyse, genomische *in situ* hybridisatie (GISH) en fluorescentie *in situ* hybridisatie (FISH). Bij het RFLP onderzoek werden voor de herkenning van individuele tomatenchromosomen chromosoomspecifieke probes van tomaat gebruikt.

Voor de productie van BC₁ nakomelingen waarmee een volledige serie van 12 monosome addities van tomatenchromosomen in een tetraploïde aardappel zou kunnen

worden gerealiseerd, werden drie verschillende somatische fusiehybriden (C31-17-5, C31-17-24 en C31-17-51) als moeders gebruikt in kruisingen met vier tetraploïde aardappelklonen, te weten de rassen Katahdin en Frieslander en de klonen 6704-1 en AM 66.42 (hoofdstuk 2). Uit deze kruisingen werden verschillende BC₁ nakomelingschappen verkregen. Hiervan werden zes BC₁ planten (6739, 2001, 2002, 2003, 2004 en 2005) geanalyseerd met behulp van RFLP en GISH analyses. Hoewel preferentiële eliminatie van soortvreemde tomatenchromosomen optrad, werd er toch regelmatig een variabel aantal tomatenchromosomen (6-11) in deze genotypen gevonden. Een belangrijk resultaat was dat in de zes BC₁ nakomelingen alle 12 mogelijke tomatenchromosomen aanwezig waren, alhoewel ze over verschillende genotypen verdeeld waren.

Door terugkruising van drie van deze BC₁ planten met tetraploïde aardappel werden in totaal 97 BC₂ nakomelingen verkregen die met behulp van RFLP en GISH werden geanalyseerd (hoofdstuk 3). Het aantal soortvreemde tomatenchromosomen in deze BC₂ planten was afgenomen en varieerde van 0 tot 6. De gemiddelde overdracht van tomatenchromosomen varieerde van 1.7 tot 3.4 in de verschillende populaties. In het algemeen werd een veel hogere mate van chromosoomoverdracht geconstateerd wanneer het soortvreemde tomatenchromosoom in disome conditie verkeerde. Een statistische analyse van de vrouwelijke chromosoomoverdracht gaf aan dat de kans op overdracht voor elk individueel tomatenchromosoom niet even groot was. Een belangrijk resultaat van het onderzoek aan de BC₂ nakomelingen was dat van de 12 mogelijke monosome addities zeven konden worden geïdentificeerd, te weten voor de chromosomen 1, 2, 4, 6, 8, 10 en 12. Dit toonde duidelijk aan dat het, ondanks het probleem van selectieve eliminatie van de soortvreemde tomatenchromosomen, mogelijk is om genotypen te selecteren waarin de soortvreemde chromosomen stabiel aanwezig zijn en bovendien via de gameten naar de nakomelingen kunnen worden overgedragen.

Behalve monosome addities werden bij de RFLP en GISH analyses ook disome addities gedetecteerd (hoofdstuk 4). Opvallend was dat in BC₂ plant 2101-1 één van de chromosomen van zo'n disome additie veel kleiner was dan het andere. Een gedetailleerde analyse van de microsporogenese toonde aan dat in het pachyteen stadium homologe paring optrad tussen het grote en het kleine tomatenchromosoom.

Morfologisch kon dit chromosoom worden geïdentificeerd als chromosoom 10 van tomaat, wat overeen kwam met de RFLP analyse met chromosoomspecifieke probes. Het kleinere chromosoom van dit homologe paar bleek te zijn ontstaan door een deletie van het heterochromatische deel van de lange arm van chromosoom 10 van tomaat. Door middel van gedetailleerde analyse van de microsporogenese kon worden aangetoond dat vroegtijdige disjunctie en deling van het soortvreemde chromosoompaar de oorzaak was van het ontstaan van disome addities in dit materiaal. Door het hierbeschreven onderzoek kon worden aangetoond dat de pachyteen chromosomen van tomaat met behulp van GISH duidelijk herkend kunnen worden, zelfs in een polyploid genotype, en dat deleties goed gekarakteriseerd kunnen worden. Bovendien werd een BC₃ populatie bestaande uit 96 planten gemaakt door terugkruising van de disome additie met pollen van een tetraploid aardappelgenotype en gekarakteriseerd met RFLP en GISH. Hiermee kon de vrouwelijke transmissie van zowel de normale als de afwijkende homologe chromosomen worden vastgesteld.

Om meer inzicht te krijgen in de homoeologe paring en overkruising tussen aardappel- en tomatenchromosomen, werd onderzoek verricht aan een somatische fusie hybride (C31-17-51) met twee translocatie-chromosomen en twee genotypen (2103-1 en 2301-2) met monosome addities van respectievelijk tomatenchromosoom 1 en 8 (hoofdstuk 5). Dit onderzoek leverde duidelijk bewijs op voor de aanwezigheid van een reciproke translocatie in de somatische fusie hybride. De vorming van quadrivalenten werd veroorzaakt door autosyndetische paring. Door het gebrek aan kennis over deze translocatie zou de hoge mate van paring, te weten 86%, ten onrechte kunnen worden aangezien voor allosyndetische paring. Analyse van de chromosoomparing en chiasmavorming in de monosome addities duidde op een zeer lage frequentie van homoeologe paring, namelijk 1,3 en 1,1% voor respectievelijk chromosoomaddities 1 en 8. Chiasmavorming tussen homoeologe chromosomen bleek voor te komen in 0,8% van de geanalyseerde meiotische cellen in de monosome additie voor chromosoom 8. Alhoewel de mate van homoeologe paring laag is, bestaan er zeker mogelijkheden voor de introgressie van chromosomaal DNA van tomaat in het genoom van de aardappel.

Résumé

Le transfert de chromosomes et de gènes au delà de barrières d'incompatibilité interspécifiques peut être utile non seulement pour l'introggression de caractères désirés mais aussi pour des études de génétique fondamentale. La démonstration réussie de l'hybridation de pomme de terre (*Solanum tuberosum*) et de tomate (*Lycopersicon esculentum*) par l'intermédiaire de fusion de protoplastes (1978) a permis d'introggresser des chromosomes et des gènes d'une espèce à une autre. Néanmoins, les perspectives d'introduction de chromosomes de tomate dans le génome de pomme de terre ne sont devenues réalisables qu'après rétrocroisement de l'hybride pomme de terre (+) tomate avec une pomme de terre tétraploïde (1994). Malgré cette prouesse, le problème d'élimination sélective des chromosomes étrangers de tomate des hybrides issus de fusion somatique et des générations suivantes issues de rétrocroisement ("backcross", BC), ainsi que le comportement des chromosomes étrangers de tomate, soulèvent les questions suivantes: (i) tout en considérant l'élimination sélective des chromosomes étrangers de tomate, est-il possible d'établir des séries complètes de chromosomes étrangers de tomate dans un environnement génomique de pomme de terre?, (ii) les chromosomes étrangers de tomate introgressés conservent-ils leur intégrité structurelle?, (iii) les chromosomes étrangers de tomate peuvent-ils être transmis à la descendance au travers des gamètes, et quelle en est la fréquence?, (iv) si des changements structurels des chromosomes étrangers introgressés existent, peuvent-ils être identifiés et caractérisés? (v) les chromosomes de tomate et de pomme de terre s'apparient-ils de façon homéologue et recombinent-ils?

Afin de répondre à ces cinq questions, nous avons essayé de créer le matériel végétal approprié au cours de cette étude. Pour cela, les constitutions chromosomiques des hybrides issus de fusion somatique ainsi que de leurs descendants issus de rétrocroisements avec la pomme de terre BC₁, BC₂ et BC₃, ont été analysés à l'aide du polymorphisme de longueur des fragments de restriction ("restriction fragment length polymorphism", RFLP) en utilisant des sondes spécifiques de chromosomes de tomate, d'hybridation *in situ* génomique ("genomic *in situ* hybridisation", GISH), et d'hybridation *in situ* à fluorescence ("fluorescence *in situ* hybridisation", FISH).

Afin de produire des descendance BC₁ ayant le potentiel complet pour la création des 12 additions monosomiques possibles de chromosomes de tomate dans un environnement de pomme de terre tétraploïde, trois hybrides hexaploïdes de fusion somatiques, C31-17-5, C31-17-24 et C31-17-51, ont été utilisés comme parents femelles dans les croisements avec quatre clones de pomme de terre tétraploïdes, cultivar Katahdin, cultivar Frieslander, 6704-1 et AM66.42 (chapitre 2). Plusieurs descendance BC₁ ont été obtenues après croisements. Parmi celles-ci, six plantes BC₁, 6739, 2001, 2002, 2003, 2004, et 2005 ont été analysées à l'aide de RFLP et de GISH. A cause de l'élimination préférentielle de chromosomes de tomate, un nombre variable de chromosomes de tomate (6-11) était présent dans ces génotypes. Néanmoins, un résultat important est le fait que les 12 différents chromosomes de tomate étaient présents dans ces six descendance BC₁ mais dans des génotypes différents.

Par rétrocroisement de trois de ces plantes BC₁ avec des pommes de terre tétraploïdes, 97 plantes BC₂ ont été obtenues et analysées à l'aide de RFLP et de GISH (chapitre 3). Le nombre de chromosomes étrangers de tomate au sein de ces plantes BC₂ varie de 0 à 6. Le taux moyen de transmission de chromosomes de tomate dans les différentes populations varie entre 1.7 et 3.4. En général, un taux de transmission nettement supérieur a été observé lorsque les chromosomes étrangers de tomate dans les plantes BC₁ étaient dans des conditions disomiques. Une équiprobabilité de transmission par le parent femelle des différents chromosomes de tomate n'a pas pu être démontrée statistiquement. Un résultat important révélé par l'analyse des plantes BC₂, est l'identification de seulement sept additions monosomiques sur les douze possibles, chromosomes 1, 2, 4, 6, 8, 10 et 12. Ceci indique clairement que malgré les problèmes d'élimination sélective des chromosomes étrangers de tomate, il est possible de sélectionner des génotypes qui conservent les chromosomes étrangers de façon stable et les transmettent au travers des gamètes à leur descendance.

En plus des additions monosomiques, une addition disomique a également été détectée grâce à des analyses de RFLP et GISH (chapitre 4). Il est à noter qu'un des chromosomes de cette paire dans la plante BC₂ 2101-1, était beaucoup plus petit que

l'autre. Une analyse détaillée de la microsporogénèse indique qu'au stade pachytène le grand et le petit chromosome de tomate s'apparient comme des homologues, et une analyse morphologique et par RFLP avec des sondes spécifiques, a identifié ce chromosome comme étant le chromosome 10 de tomate. L'analyse morphologique a permis de conclure qu'une délétion de la partie hétérochromatique du bras long du chromosome 10 de tomate a donné lieu au plus petit de cette paire homologue. Une analyse détaillée de microsporogénèse indique qu'une disjonction précoce et une division de la paire de chromosomes étrangers est à l'origine de l'addition disomique dans ce matériel. D'après cette étude, il paraît évident que les chromosomes de tomate au stade pachytène peuvent être identifiés à l'aide d'analyses de GISH, et les délétions caractérisées et cela même dans un génotype polyploïde. De plus, une population BC3 comprenant 96 plantes a été obtenue et caractérisée à l'aide d'analyses de RFLP et de GISH, après rétrocroisement de l'addition disomique avec une pomme de terre tétraploïde prise comme parent mâle. Par conséquent, la transmission femelle des homologues normaux et des homologues aberrants a pu être déterminée.

Afin de clarifier l'appariement homéologue et la recombinaison entre les chromosomes de pomme de terre et de tomate, un hybride obtenu par fusion somatique, C31-17-5, ayant deux chromosomes transloqués, et deux génotypes, 2103-1 et 2103-2, ayant des additions monosomiques de chromosomes de tomate, respectivement chromosome 1 et 8, ont été étudiés (chapitre 5). La présence d'une translocation réciproque dans l'hybride de fusion somatique a été démontrée. Par conséquent, la formation quadrivalente impliquant des chromosomes de pomme de terre et de tomate était uniquement due à un appariement autosyndétique. En l'absence de connaissance de cette translocation, le fort pourcentage d'appariement, 86.0%, aurait pu être confondu avec un appariement allosyndétique. Une analyse de l'appariement des chromosomes et de la formation des chiasmas dans les additions monosomiques indique une fréquence très faible d'appariement homéologue, 1.3 et 1.1% pour les additions de chromosomes 1 et 8 respectivement. La formation de chiasmas entre chromosomes homéologues a été estimée dans 0.8% des cellules méiotiques étudiées dans l'addition monosomique du chromosome 8. Malgré la faible fréquence d'appariement homéologue l'introgression d'ADN chromosomique de tomate dans le génome de pomme de terre présente un réel intérêt.

Resum

Transferir cromosomes i gens a través de límits intergenèrics no només pot ser útil per a introgressar caràcters desitjats sinó també per a portar a terme estudis de genètica fonamental. La demostració magistral d'hibridació de patata (*Solanum tuberosum*) i tomàquet (*Lycopersicon esculentum*) per mitjà de fusió de protoplastes l'any 1978, va crear el potencial per introgressar cromosomes i gens d'un gènere a l'altre. Malgrat tot, els prospectes d'afegir cromosomes de tomàquet dins del genoma de patata només esdevingeren reals quan un dels esmentats híbrids de fusió somàtica es va poder retrocreuar amb patata tetraploid l'any 1994. Malgrat aquesta proesa, el problema d'eliminació selectiva dels cromosomes aliens de tomàquet en els híbrids de fusió somàtica i en les subseqüents generacions de retrocreuament també anomenades generacions de "backcross (BC)", així com el comportament dels esmentats cromosomes aliens dins del genoma de patata, van suggerir les següents qüestions: (i) en vistes de l'eliminació selectiva dels cromosomes de tomàquet, és possible establir la sèrie completa d'adicions de cromosomes de tomàquet dins del genoma de patata? (ii) mantenen els cromosomes de tomàquet introgressats la seva integritat estructural? (iii) es poden transmetre tots els cromosomes de tomàquet a través dels gamets a la descendència, i si aquest fos el cas, en quina freqüència? (iv) en cas de que algun cromosoma introgressat patís algun canvi estructural, és possible identificar-l'ho i caracteritzar-l'ho? (v) s'aparellen i es recombinen els cromosomes homoeòlegs de patata i tomàquet?

Per tal de contestar les esmentades preguntes, en aquest treball de recerca s'intentà de crear el material vegetal apropiat. En aquest respecte, la constitució cromosòmica de diferents híbrids de fusió somàtica i el dels seus descendents resultants dels retrocreuaments amb patata tetraploid (BC₁, BC₂, i BC₃) es van analitzar per mitjà d'una combinació de: "restriction fragment length polymorphism (RFLP)" tot utilitzant sondes específiques de cromosomes de tomàquet, "genomic *in situ* hybridisation (GISH)" i "fluorescence *in situ* hybridisation (FISH)".

Pel tal de produir progenie BC₁ amb el potencial complet per a poder establir les 12 possibles adicions monosòmiques de tomàquet al genoma tetraploid de patata, es van utilitzar com a progenitors materns tres híbrids hexaploids de fusió somàtica:

C31-17-5, C31-17-24, i C31-17-51 en creuaments amb quatre clons de patata tetraploids: cultivars Katahdin i Frieslander, i els clones 6704-1 i AM 66.42 (capítol 2). A partir d'aquests retrocreuaments es van obtenir varis genotips BC₁. D'entre ells, sis plantes: 6739, 2001, 2002, 2003, 2004 i 2005, es van analitzar mitjançant RFLP i GISH. Degut a l'eliminació preferencial dels cromosomes de tomàquet, el seu nombre va variar entre 6 i 11 en les diferents genotips. Un resultat important va ser que, malgrat en diferent genotips, tots els 12 diferents cromosomes de tomàquet estaven presents en aquest genotips BC₁.

Retrocreuant tres d'aquests genotips BC₁ amb patata tetraploid es van obtenir un total de 97 genotips BC₂ els quals es van analitzar mitjançant RFLP i GISH (capítol 3). El nombre de cromosomes de tomàquet en aquests genotips BC₂ va variar entre 0 i 6. La freqüència promig de transmissió de cromosomes de tomàquet entre diferents poblacions va variar entre 1.7 i 3.4. Generalment, es va observar una freqüència de transmissió molt més alta per aquells cromosomes que estaven presents en condició disòmica en els progenitors BC₁. Una anàlisi estadística de la freqüència de transmissió a través del parental matern no va poder provar una mateixa freqüència per als diferents cromosomes de tomàquet. Un resultat important d'aquesta investigació fou que, d'entre els 12 possibles tipus d'adicions monosòmiques, se'n van identificar set; pels cromosomes 1, 2, 4, 6, 8, 10 i 12. Això va indicar clarament que malgrat el problema d'eliminació selectiva dels cromosomes de tomàquet, és possible seleccionar genotips que retenen els cromosomes aliens en condició estable i que els poden transmetre a través dels gamets a les generacions posteriors.

A més de les adicions monosòmiques, se'n va detectar una de disòmica mitjançant anàlisi amb RFLP i GISH (capítol 4). S'observà que un dels cromosomes d'aquesta parella en el genotip BC₂, 2101-1, era remarcablement força més petit que l'altre. Una anàlisi detallada en el procés de microsporogènesi indicà que a l'estat de paquitene els dos cromosomes de tomàquet (petit i normal) s'aparellaren com a homòlegs, i morfològicament es van identificar com el cromosoma 10 de tomàquet, la qual cosa fou concurrent amb els resultats de les anàlisi amb RFLP utilitzant sondes específiques per als cromosomes de tomàquet. A més, aquesta anàlisi també va aportar evidències sobre l'origen d'adicions disòmiques en aquest tipus de material el qual fou atribuït a una separació i divisió precoces dels cromosomes de tomàquet. Aquest

estudi va demostrar que els cromosomes de tomàquet a l'estat de paquitene es poden identificar clarament utilitzant la tècnica de GISH inclús estan incorporats dins d'un genotip poliploid de patata, i que alteracions morfològiques poden ser caracteritzades. A més, es va crear una població BC₃ de 96 plantes retroceuant l'adició disòmica amb patat tetraploid. L'esmentada població s'analitzà mitjançant RFLP i GISH, la qual cosa va oferir la possibilitat de determinar la freqüència de transmissió materna d'ambdós cromosomes homòlegs, normal i mutant, a la subseqüent generació.

Per tal d'aclarir la qüestió d'aparellament homoeòleg i recombinació entre els cromosomes de patata i tomàquet, es van estudiar: un híbrid de fusió somàtica, C31-17-51, el qual posseeix dos cromosomes translocats, i dos genotips de patata tetraploid amb adicions monosòmiques per als cromosomes 1 (clon 2103-1) i 8 (clon 2301-2) (capítol 5). Aquest estudi va evidenciar que les translocacions presents en l'híbrid de fusió somàtica eren recíproques. Com a freqüència, la formació de quatrivalents involucrant cromosomes de patata i tomàquet fou deguda única i exclusivament a aparellament autosindètic. En l'absència del coneixament de l'existència d'aquestes translocacions, l'alta freqüència d'aparellament (86,0%) entre els cromosomes translocats i els seus respectius homòlegs de patata i tomàquet, es podria haver malinterpretat com a aparellament allosindètic. Un estudi en l'aparellament dels cromosomes i en la formació de quiasmes en les adicions monosòmiques, va demostrar una freqüència molt baixa d'aparellament homoeòleg, 1.3 i 1.1% per a les adicions dels cromosomes 1 i 8 respectivament. La formació de quiasmes entre cromosomes homoeòlegs es va determinar en una freqüència del 0,8% de les cel·lules meiótiques analitzades en l'adició monosòmica pel cromosoma 8.

Els resultats d'aquest treball de recerca van demostrar de manera conclüent que, malgrat la baixa freqüència d'aparellament entre cromosomes homoeòlegs de patat i tomàquet, és possible introgressar ADN cromosòmic de tomàquet al genoma de patata.

Resumen

Transferir cromosomas y genes a través de límites intergenéricos no solamente puede ser útil para introgresionar caracteres deseados sino también para llevar a cabo estudios fundamentales de genética. La clara demostración de la hibridación de patata (*Solanum tuberosum*) y tomate (*Lycopersicon esculentum*) mediante fusión de protoplastos en el año 1978, creó el potencial para introgresionar cromosomas y genes de un género en el otro. Sin embargo, las expectativas para añadir cromosomas de tomate dentro del genomio de patata no se hicieron realidad hasta que los híbridos de fusión somática se pudieron retrocruzar con patata tetraploide en el año 1994. A pesar de esto, el problema de eliminación selectiva de los cromosomas de tomate en los híbridos de fusión somática y en las siguientes generaciones de retrocruzamiento, también llamadas generaciones de “backcross (BC)”, así como el comportamiento de los cromosomas de tomate dentro del genomio de patata, sugirieron las siguientes preguntas: (i) teniendo en cuenta la eliminación selectiva de los cromosomas de tomate ¿sería posible establecer la serie completa de adiciones de cromosomas de tomate dentro del genomio de patata? (ii) ¿mantienen los cromosomas introgresionados de tomate su integridad estructural? (iii) ¿se pueden transmitir sexualmente todos los cromosomas de tomate a través de los gametos a la descendencia, y si así fuese, con qué frecuencia? (iv) en caso de que algún cromosoma introgresionado sufra algún cambio estructural ¿sería posible identificarlo y caracterizarlo? (v) ¿se aparean y se recombinan los cromosomas homoeólogos de patata y tomate?

Para contestar estas cinco preguntas, en este trabajo de investigación se intentó crear el material vegetal adecuado. A este respecto, la constitución cromosómica de distintos híbridos de fusión somática y el de sus descendientes resultantes del retrocruzamiento con patata tetraploide (BC₁, BC₂ y BC₃) se analizó mediante una combinación de: “restriction fragment length polymorphism (RFLP)”, utilizando sondas específicas de cromosomas de tomate, “genomic *In Situ* hybridization (GISH)” y “fluorescence *In Situ* hybridization (FISH)”.

Con el fin de producir progenie BC₁ con el potencial completo para establecer las 12 posibles adiciones monosómicas de tomate en el genomio tetraploide de patata se utilizaron como progenitores maternos tres híbridos de fusión somática: C31-17-5,

C31-17-24 y C31-17-51, en cruzamientos con cuatro clones de patata tetraploide: cultivares Katahdin y Frieslander y los clones 6704-1 y AM66.42 (capítulo 2). A partir de estos retrocruzamientos se obtuvieron varios genotipos BC₁. De entre ellos, seis plantas: 6739, 2001, 2002, 2003, 2004 y 2005 se analizaron mediante RFLP y GISH. Debido a la eliminación preferencial de los cromosomas de tomate, el número de estos varió entre 6 y 11 en los diferentes genotipos, destacando que, aunque en diferentes genotipos, todos los 12 diferentes cromosomas del tomate estaban presentes en estos genotipos BC₁.

Retrocruzando tres de estos genotipos BC₁ con patata tetraploide se obtuvieron un total de 97 genotipos BC₂, los cuales se analizaron mediante RFLP y GISH (capítulo 3). El número de cromosomas de tomate en estos genotipos BC₂ varió entre 0 y 6. La frecuencia promedio de transmisión de cromosomas de tomate entre diferentes poblaciones varió entre 1,7 y 3,4. Generalmente, se observó una frecuencia de transmisión mucho más alta para aquellos cromosomas que estaban presentes en condición disómica en los progenitores BC₁. Un análisis estadístico de la frecuencia de transmisión a través del parental materno no pudo demostrar la misma frecuencia de transmisión para todos los cromosomas de tomate. Resultando notablemente que de entre los 12 posibles tipos de adiciones monosómicas, se identificaron siete para los cromosomas 1, 2, 4, 6, 8, 10, y 12. Este resultado indicó que a pesar de la eliminación selectiva de los cromosomas de tomate, es posible seleccionar genotipos que retienen los cromosomas de tomate en condición estable y que los pueden transmitir a través de los gametos a las generaciones posteriores.

Además de las adiciones monosómicas se detectó una adición disómica mediante análisis con RFLP y GISH (capítulo 4). Se observó que uno de los cromosomas de esta pareja en el genotipo BC₂, 2101-1, era bastante más pequeño que el otro. Un análisis detallado en el proceso de microesporogénesis indicó que en el estado de paquitene los dos cromosomas de tomate (pequeño y normal) se aparearon como homólogos, y se identificaron morfológicamente como el cromosoma 10 de tomate, lo cual era concomitante con los resultados de los RFLP utilizando sondas específicas para los cromosomas de tomate. Además, este análisis también aportó evidencias sobre el origen de las adiciones disómicas en este tipo de material, lo cual fue atribuido a una separación y división precoces de los cromosomas de tomate. Este

estudio demostró que los cromosomas de tomate en estado de paquitene se pueden identificar claramente utilizando la técnica de GISH incluso estando incorporados dentro de un genotipo poliploide de patata, y que alteraciones morfológicas se pueden caracterizar. Además, se creó una población BC₃ de 96 plantas retrocruzando la adición disómica con patata tetraploide. Esta población se analizó mediante RFLP y GISH, lo cual ofreció la posibilidad de determinar la frecuencia de transmisión materna de los dos cromosomas homólogos, normal y mutante, a la siguiente generación.

Con el fin de aclarar la cuestión del apareamiento y recombinación entre los cromosomas de patata y tomate, se estudiaron un híbrido de fusión somática, C31-17-51, el cual posee dos cromosomas translocados, y dos genotipos de patata con adiciones monosómicas para los cromosomas 1 (clon 2103-1) y 8 (clon 2301-2) (capítulo 5). Este estudio demostró que las translocaciones presentes en el híbrido de fusión somática eran recíprocas. Como consecuencia, la formación de cuatrivalentes involucrando cromosomas de patata y tomate fueron debidas única y exclusivamente a apareamiento autosindético. En ausencia del conocimiento de la existencia de estas translocaciones, la alta frecuencia de apareamiento (86%) entre los cromosomas translocados y sus homólogos de patata y tomate, se podría haber malinterpretado como apareamiento alosindético. El estudio del apareamiento de los cromosomas y la formación de quiasmas en la adiciones monosómicas, demostró una frecuencia muy baja de apareamiento homoeólogo, 1,3 y 1,1% para las adiciones de los cromosomas 1 y 8, respectivamente. La formación de quiasmas entre cromosomas homoeólogos se determinó en una frecuencia del 0,8% de las células meióticas analizadas en la adición monosómica para el cromosoma 8.

Los resultados del presente trabajo de investigación demostraron de forma concluyente que, a pesar de la baja frecuencia de apareamiento homoeólogo entre cromosomas de patata y tomate, es posible de introgresionar ADN cromosómico de tomate dentro del genomio de patata.

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Curriculum Vitae

Francesc Garriga Calderé was born on 15 September 1961 in Manresa, Catalonia (Spain). His early schooling was in the same city of Manresa. It was during his secondary school he got interested in genetics. Wanting to do something to improve the crops growing in the garden of his family pushed him to study agriculture. From 1987 to 1992 he studied for his B.Sc. degree at the “Escola Superior d’Agricultura de Barcelona” which he combined with a full time job. While finishing the studies, he went to the Department of Plant Breeding of the Wageningen Agricultural University to carry out the research project required to obtain the degree and got the opportunity of studying for M.Sc. (Crop Science), a 17 month course which he completed in 12 month, with distinction, in 1994. During this period he was fascinated with plant chromosomes, meiosis in polyploids and DNA. He got the opportunity of joining the cytogenetics group to persuade the Ph.D. programme, financially supported by the Dutch Potato Association (NAA, Nationale Aardapel Associatie). At present he is working as a Post-Doctoral researcher in genetic mapping of dicot plants, as a part of the European Union Project at the “IRTA, Institut de Recerca i Tecnologia Agroalimentàries” in Cabriels, Barcelona (Spain).

Publications

- Garriga-Calderé F, Huigen DJ, Jacobsen E and Ramanna (Submitted)** Prospects for introgressing tomato chromosomes into the potato genome: an assessment through GISH analysis
- Garriga-Calderé F, Huigen DJ, Jacobsen E and Ramanna (Submitted)** Origin of an alien disomic addition with an aberrant homologue of chromosome 10 of tomato and its meiotic behaviour in a potato background revealed through GISH
- Garriga-Calderé F, Huigen DJ, Angrisano A, Jacobsen E and Ramanna (1998)** Transmission of alien tomato chromosomes from BC₁ to BC₂ progenies derived from backcrossing potato (+) tomato fusion hybrids to potato: the selection of single additions for seven different tomato chromosomes. *Theor Appl Genet* 96: in press
- Garriga-Calderé F, Huigen DJ, Filotico F, Jacobsen E and Ramanna MS (1997)** Identification of alien chromosomes through GISH and RFLP analysis and the potential for establishing potato lines with monosomic additions of tomato chromosomes. *Genome* 40 (5): 666-673
- Marjo J. de Jeu and **Francesc Garriga Calderé (1997)** Retarded embryo growth and early degeneration of sporophytic tissue are associated with embryo abortion in the interspecific cross *Alstroemeria pelegrina* L. x *Alstroemeria aurea*. *Can J Bot* 75:916-924
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