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Transmission of alien tomato chromosomes from BC_1 to BC_2 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 BC_1 genotypes of potato (+) tomato fusion hybrids to different tetraploid potato pollinators, BC_2 populations were produced. A combined total of 97 BC_2 plants from three BC_2 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 BC_1 parent ranged from 0 to 6, and the average number of different alien chromosomes transmitted per BC_2 plant varied between 1.7 and 3.4 in the different populations. This variation corresponded to the chromosome constitution of the individual BC_1 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 BC_1 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 BC_2 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.

Key words *Solanum tuberosum* · *Lycopersicon esculentum* · Protoplast fusion · Monosomic additions · GISH · FISH

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 which 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)

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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 BC_1 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 BC_1 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 BC_1 plants and to evaluate whether all alien tomato chromosomes were present and can be introduced into potato. To this end, new BC_1 progenies were produced by using additional individual fusion hybrids and potato pollinators (Garriga-Calderé et al. 1997). RFLP analyses of these BC_1s showed that all the tomato chromosomes were present at the BC_1 level. In addition, these new BC_1 progenies were successfully backcrossed to several tetraploid genotypes of potato and BC_2 progeny populations were obtained.

The aims of the present investigation were: (1) to characterise BC_2 populations in which aneuploids had originated from the sexual transmission of alien tomato chromosomes; (2) to analyse the probability of female transmission of the alien tomato chromosomes from different BC_1 to BC_2 progeny plants, and (3) to select potato genotypes with single additions of tomato chromosomes.

Materials and methods

Three BC_1 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 BC_1s were successfully crossed with eight different tetraploid potato pollen parents, viz., a breeding clone AM66.42, cv Desiréé, 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 BC_1 female parents, three sets of populations of BC_2 progenies were analysed and the details of their origin are illustrated in Fig. 1. The sets of three BC_2 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, BC_1 plants, pollinators and the BC_2 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 BC_1s 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 BC_1 6739 (chromosome 7 was absent). Chromosomes 5 and 12 were not detected by RFLP analyses in BC_1 2003, although GISH revealed 12 tomato chromosomes indicating that two alien chromosomes were in a disomic condition and that they regularly formed two bivalents at metaphase-I of meiosis (see Fig. 2). Likewise, chromosome 3 was not detected through RFLP analysis in BC_1 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 BC_1s to tetraploid potato, the BC_2 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 hybridisation were according to Kreike et al. (1990). Polymorphisms were found after the DNA was digested with either *Eco*RI or *Eco*RV. For a reliable chromosome identification, the tomato chromosome-specific probes were selected on the basis that: (1) the probe showed clear polymorphism between the tomato and potato parents; (2) the polymorphic bands of both species were clearly identifiable in the fusion hybrid; (3) the polymorphic band of tomato was absent in the pollen parent(s) used in backcrosses, and (4) the polymorphic band unequivocally identified the particular tomato chromosome in the BC_1 and BC_2 progenies (see Fig. 3).

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 BC_1s 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-carmine 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 phosphate 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 of 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). 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 BC_1 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 four times for 1 h in 4 × SSC plus 0.5% among 20. The slides were then dehydrated in an ethanol series.

The rDNA probe was pTA71 which contains the 5.8*s*-18*s*-26*s* ribosomal genes (Gerlach and Bedbrook 1979) and which was labelled with biotin following a standard nick-translation protocol

(Boehringer Mannheim). The hybridisation mixture, hybridisation conditions and stringency washings were similar to those mentioned above. A three-step detection using streptavidin coupled to Cy-3 (Jackson Immuno Research Laboratories), and biotinylated-anti-streptavidin (Vector Laboratories) was according to the manufacturer. The chromosome spreads were counterstained with 2 µg/ml of DAPI (4′6-diamidino-2-phenylindole) and mounted in 10 µl of anti-fade. Selected chromosome spreads were photographed on 400 iso-colour negative film using an Axiophot microscope equipped with UV light and the appropriate filter block.

Statistical analysis of alien tomato-chromosome transmission

The frequencies of female transmission within and among the BC$_2$ 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 BC$_2$ progenies

A total of 97 plants, belonging to three different BC$_2$ populations (Fig. 1), were evaluated for the presence or absence of alien tomato chromosomes through RFLP analysis. These three populations were derived from BC$_1$s 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 BC$_1$s, as well as in the BC$_2$ progenies, was that there were four intact genomes of potato together with a variable number of alien tomato chromosomes. The BC$_1$ parent 6739 possessed 11 single alien tomato chromosomes in contrast to BC$_1$s 2003 and 2002. BC$_1$ 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. 2); whereas BC$_1$ 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 BC$_1$ 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. 2A, B). In addition the bivalents were also detected at the diakinesis and metaphase-I stages of meiosis (Figs. 2C, 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. 2D, 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. 3). Based on such analyses, the alien chromosome constitution of the BC$_2$ progenies that were characterised are presented in Table 1.

The number and frequency of BC$_2$ 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 BC$_2$ 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

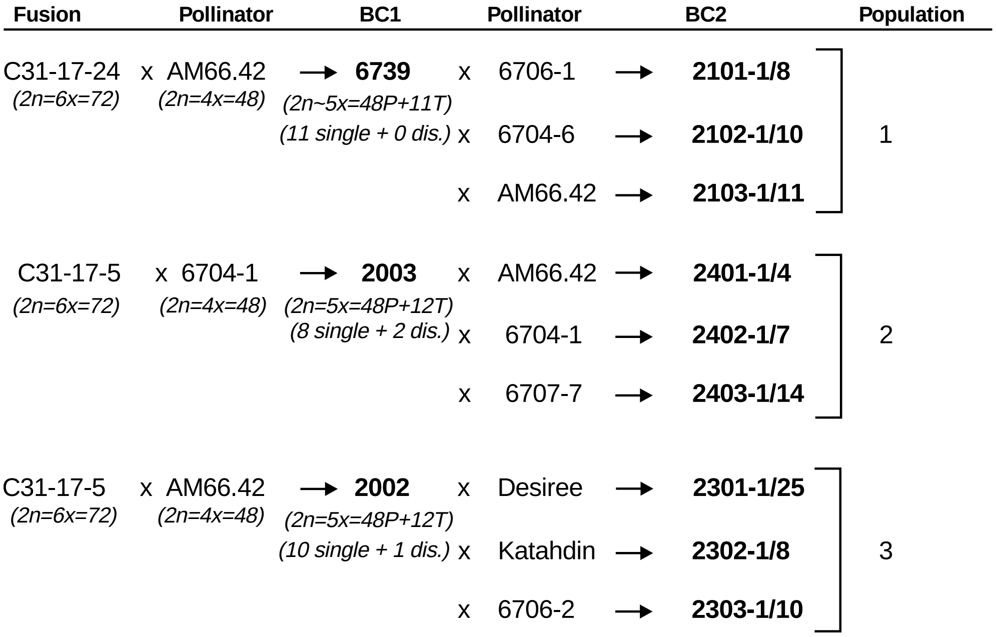
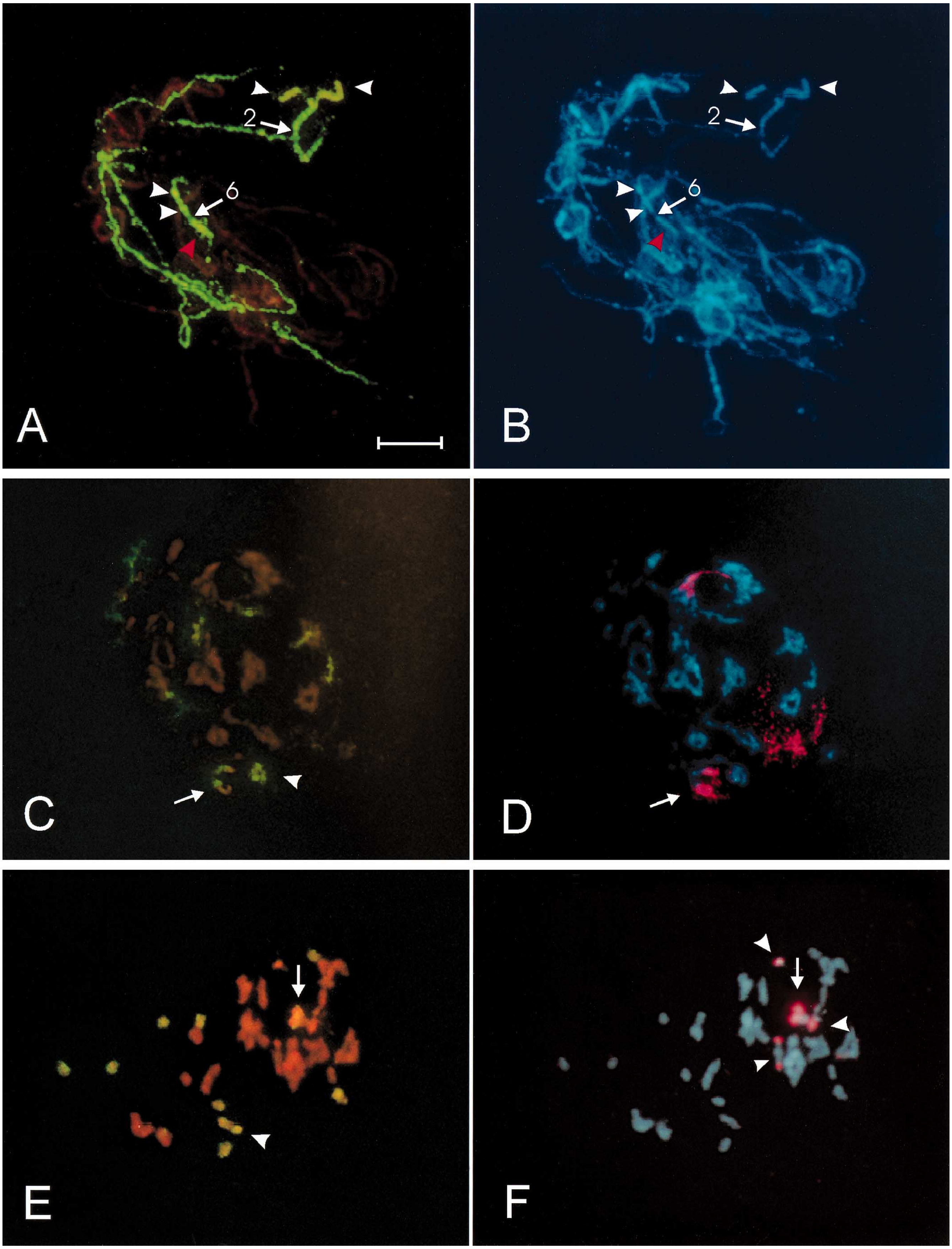


Fig. 1 Pedigree chart of the potato (+) tomato fusion hybrids and backcross progenies. Eleven alien tomato chromosomes were detected in a single condition in the BC$_1$ 6739, whereas one and two alien tomato chromosomes were detected in a disomic condition in BC$_1$s 2002 and 2003, as indicated by *1 dis.* and *2 dis.* respectively, which regularly formed bivalents at meiosis. The *column BC$_2$* indicates the code and number of plants in each BC$_2$ population


2
6
A
2
6
B
C
D
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F

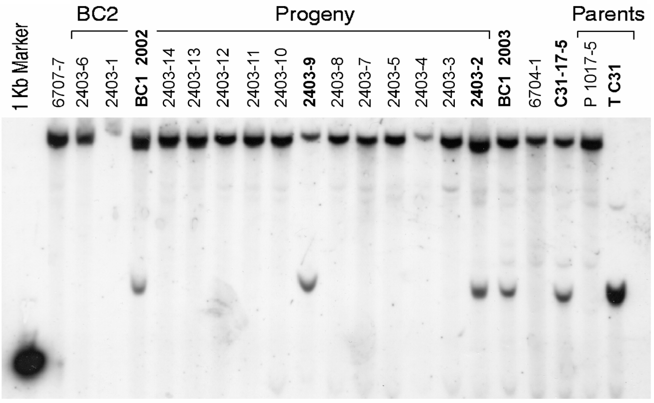

Fig. 3 A representative autoradiogram of a Southern blot illustrating the detection of an alien tomato chromosome addition through RFLP analysis. The tomato parent, T C31 and the potato parent, P1017-5 show clear polymorphism. Both bands are clearly present in the fusion hybrid C31-17-5 and in the two BC_1s, 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 BC_2 progenies only two genotypes, 2403-2 and 2403-9, posses the polymorphic band of the probe TG46 which identifies chromosome 11 of tomato

tomato chromosome 1 and not a single BC_2 plant possessed tomato chromosome 9. This means that among the 11 individual alien chromosomes of the BC_1 parent 6739, only ten were transmitted to individual plants of this BC_2 population. The average number of transmission of tomato chromosomes to BC_2 plants in this population was 1.7. In the other two BC_2 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 BC_2 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 BC_2 progeny was 3.4 per plant (Table 1). On the other hand, only one plant did not posses 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 BC_2 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 BC_1 plants. Chromosomes present in a disomic condition in the BC_1s are expected to form bivalents during meiosis (Jacobsen et al. 1995) which should lead to their more regular 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 BC_2 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 BC_2 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 BC_2 plants with different numbers of individual tomato chromosomes clearly reflected the chromosome constitution of the BC_1 parents. In other words, it depended on whether the BC_1 parent possessed only chromosomes in a single condition as in BC_1 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 the BC_1 2002, the parent of population 3, and in BC_1 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 BC_1 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 BC_2 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,

◄

Fig. 2A–F Alien tomato chromosome constitution in BC_1 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 pTA 71 (5.8*s*-18*s*-26*s*) rDNA fluorescing *red* in **D** (*arrow*); the *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 pTA 71 (5.8*s*-18*s*-26*s*) 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

Table 1 Number and frequency of transmission of individual alien tomato chromosomes in three different BC_2 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	
BC_1[c]	11 single + no disomics		8 single + 2 disomics		10 single + 1 disomic	

[a] The alien tomato chromosome was already absent in the BC_1

[b] 0 = not transmitted

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

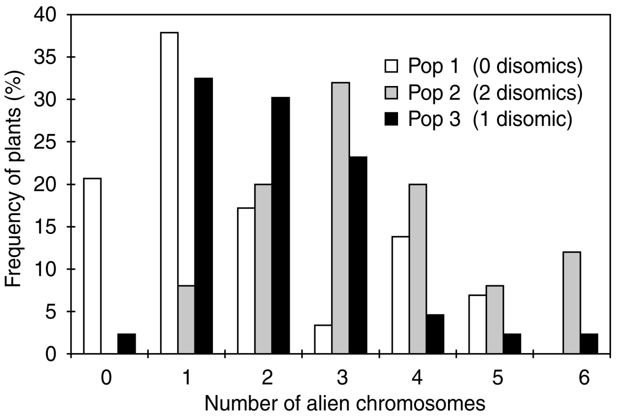

Fig. 4 Histogram of the frequency of classes for three different BC_2 populations in relation to the number of alien tomato chromosome additions per BC_2 plant. Note that there is a shift towards a higher number of chromosome additions when more chromosomes in a disomic condition (as indicated with 0, 1 and 2 disomics) were present in the BC_1 parent

the BC_2 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 BC_1 parents. Thus, in view of selecting plants with only one alien tomato chromosome addition, populations 1 (from a BC_1 with no disomics) and 3 (from a BC_1 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 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 BC_1 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. 2) in the BC_1 parent.

Probability of the female transmission of individual alien tomato chromosomes

Within the BC_2 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

Table 2 Frequency and types of single[a] additions of individual tomato chromosomes found in the three different BC_2 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 + 0 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

[a] They could be in a mono- or di-somic condition

Table 3 Testing for equality of female transmission of the different alien tomato chromosomes. In the case of disomics in BC_1 the test of equal transmission in the population 2 and 3 calculations were made by both including and excluding the disomic tomato chromosomes

Disomics in BC_1	Population	*n* classes[a]	χ^2[b]	*df*[c]	*P*[d]
Within the three BC_2 populations					
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[e]		7.72	2	0.021
4[e]	3[e]		3.75	2	0.153
8[e]	3[e]		0.33	2	0.847
9[e]	3[e]		8.78	2	0.012
10[e]	3[e]		0.23	2	0.891
11[e]	3[e]		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] Chromosome, individual chromosome tested

meiosis. Therefore, when the null-hypothesis was tested, after excluding those chromosomes in a disomic condition in the BC_1s (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 ($P = 0.898$).

Among the BC_2 populations

In this case, only those chromosomes that were present in a single condition in all three BC_1 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).

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 BC_2 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 BC_2 progenies, but also that the BC_1 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 BC_1 and BC_2 progenies had to be created through embryo-rescue techniques 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 BC_2 plants (92.8%) possessing alien chromosome additions.

There were indeed differences among the 12 individual tomato chromosomes that were transmitted to the BC_2 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 BC_1 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 BC_1s (e.g. chromosomes 2 and 6 for population 2, and chromosome 6 for population 3). The same phenomenon was found to occur in a BC_2 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 BC_1 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 BC_1 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 BC_1 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 BC_1s, 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 BC_2 progenies of populations 2 and 3 possessed a higher number of alien chromosomes than the BC_2 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. 2). 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 BC_2 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 BC_2 population of a potato (+) tomato hybrid (Jacobsen et al. 1995). Additional GISH analyses are needed to discriminate between monosomic and disomic additions in the BC_2 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 disomic 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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