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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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Abstract While characterising potato (*Solanum tuberosum*, 2n = 4*x* = 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 BC_2 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 with two alien tomato chromosomes, of which one was much smaller than the other. Analysis of chromosome pairing at the 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 the sub-telomeric repeat TGRI, showed intact telomeres and sub-telomeres 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 BC_3 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 the physical mapping of chromosome-10.

Key words *Solanum tuberosum* · *Lycopersicon esculentum* · Disomic additions · Deletion · GISH · FISH

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Introduction

Alien chromosome additions have traditionally been used for gene introgression in plants but they are also becoming increasingly useful in molecular cytogenetic studies. Some examples, among others, are: (1) to determine syntenic relationships and increase the accuracy of genetic maps (Chen et al. 1997; Suen et al. 1997), (2) molecular tagging and cloning of alien genes (Ishii et al. 1994; Potz et al. 1996), (3) physical mapping of chromosomes (Gill et al. 1996), (4) to establish chromosome-specific DNA libraries (Riera-Lizarazu et al. 1996; Ananiev et al. 1997), (5) introgression mapping (King et al. 1997), and (6) to unravel the molecular organisation of individual chromosomes or parts of chromosomes (Fransz et al. 1996; Zhong et al. 1996; HW Raines, personal communication). Because of these attractive features, interest for distant hybrids and their backcross derivatives has increased for 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).

The addition of whole, or (recombinant) parts of, chromosomes into an alien background can be useful for the 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 to that of 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), 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 the alien chromosomes have been observed. Here we describe: (1) 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, (2) the meiotic behaviour of the normal counterpart along with the mutant chromosome-10 of tomato in one and the same plant, and (3) the rate of transmission of the normal and mutant chromosomes.

Materials and methods

Plant material

The BC_2 plant 2101-1, a disomic addition possessing an aberrant homologue for the alien tomato chromosome-10, which was derived from a hexaploid tomato (+) potato somatic 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 BC_3 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 BC_3 population consisting of 96 plants was analysed through RFLP for female transmission following the same methodology as described previously (Garriga-Calderé et al. 1998). Two DNA probes, TG285 and TG303, specific for chromosome-10 of tomato, were used for detecting the presence (or absence) of this chromosome. The probes were kindly provided by Prof. S.D. Tanksley, Cornell University, N.Y., USA.

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 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 of 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 washings were the same as those described previously (Garriga-Calderé et al. 1997). Digoxigenin was detected with 20 μg/ml of anti-dig-FITC (fluorescein isothiocyanate); (Boehringer Mannheim) and 20 μg/ml of rabbit-anti-sheep-FITC (Vector Laboratory). For each detection step the slides were incubated with 100 μl of blocking buffer 1 [0.5% blocking reagent (Boehringer Mannheim) in buffer-1 (0.1 M Tris-HCl plus 0.15 M NaCl, pH 7.0)] for 30 min, incubated for 1 h with the appropriate antibody in 100 μl of blocking buffer, and washed three times in buffer-1 for 10 min at room temperature. The chromosome spreads were then counterstained with 5 μg/ml of propidium iodide and with 2 μg/ml of DAPI (4′6-diamidino-2-phenylindole) for 10 min each at room temperature and mounted in 10 μl of 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, the hybridisation mixture, conditions and stringency washings were the same to those described previously (Garriga-Calderé et al. 1998). The three-step detection was as follows; 4 μg/ml of streptavidin-Cy3 (Jackson Immuno Research Laboratories), 10 μg/ml of biotinylated-anti-streptavidin (Vector Laboratories), and then again 4 μg/ml of streptavidin-Cy3. For each detection step the slides were incubated with 100 μl of blocking buffer 1 [0.5% blocking reagent (Boehringer Mannheim) in buffer-1 (0.1 M Tris-HCl plus 0.15 M NaCl, pH 7.0)] for 30 min, incubated for 1 h with the appropriate antibody in 100 μl of blocking buffer, and washed three times in buffer-1 for 10 min at room temperature. The chromosome spreads were then counterstained with 2 μg/ml of DAPI (4′6-diamidino-2-phenylindole) and mounted in 10 μl of Vectashield (Vector Laboratories). Besides the telomeric probe, a sub-telomeric probe, TGRI, was used for the confirmation of intact chromosome ends.

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 500 dpi and the digital images were optimised for contrast and brightness using routine image-processing software.

The telomeric probe pAtT4 and the sub-telomeric probe TGRI were kindly provided by Dr. E.J. Richards, Washington University, M.O., USA, and Prof. S.D. Tanksley, Cornell University, N.Y., USA, respectively.

Results

Detection of alien additions

The disomic addition was detected in a BC_2 progeny, clone 2101-1, resulting from repeated backcrossing of

the potato (+) tomato somatic hybrid to the tetraploid potato as a male parent. Initially, the 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 the pachytene stage the size difference observed between the pair of alien chromosomes in somatic cells was confirmed (Fig. 1 B and C). The morphology of the larger (intact) chromosome at the pachytene stage (Fig. 1 C) strictly conformed to the description of pachytene chromosome-10 of tomato (Ramanna and Prakken 1967). The short arms of the bivalent were morphologically identical (Fig. 1 B) 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, occasionally with 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 order to answer this question, the preparations in which both of the alien chromosomes were identified through GISH were re-probed using the telomeric sequence, pAtT4, for FISH analysis. Both of the alien chromosomes had all the telomeres intact in the occasional univalents observed at the pachytene stage (Figs. 1 C and D). In addition, the presence of the subtelomeric sequence repeat TGRI in both 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. 1 E) at the metaphase-I stage, indicating chiasma formation.

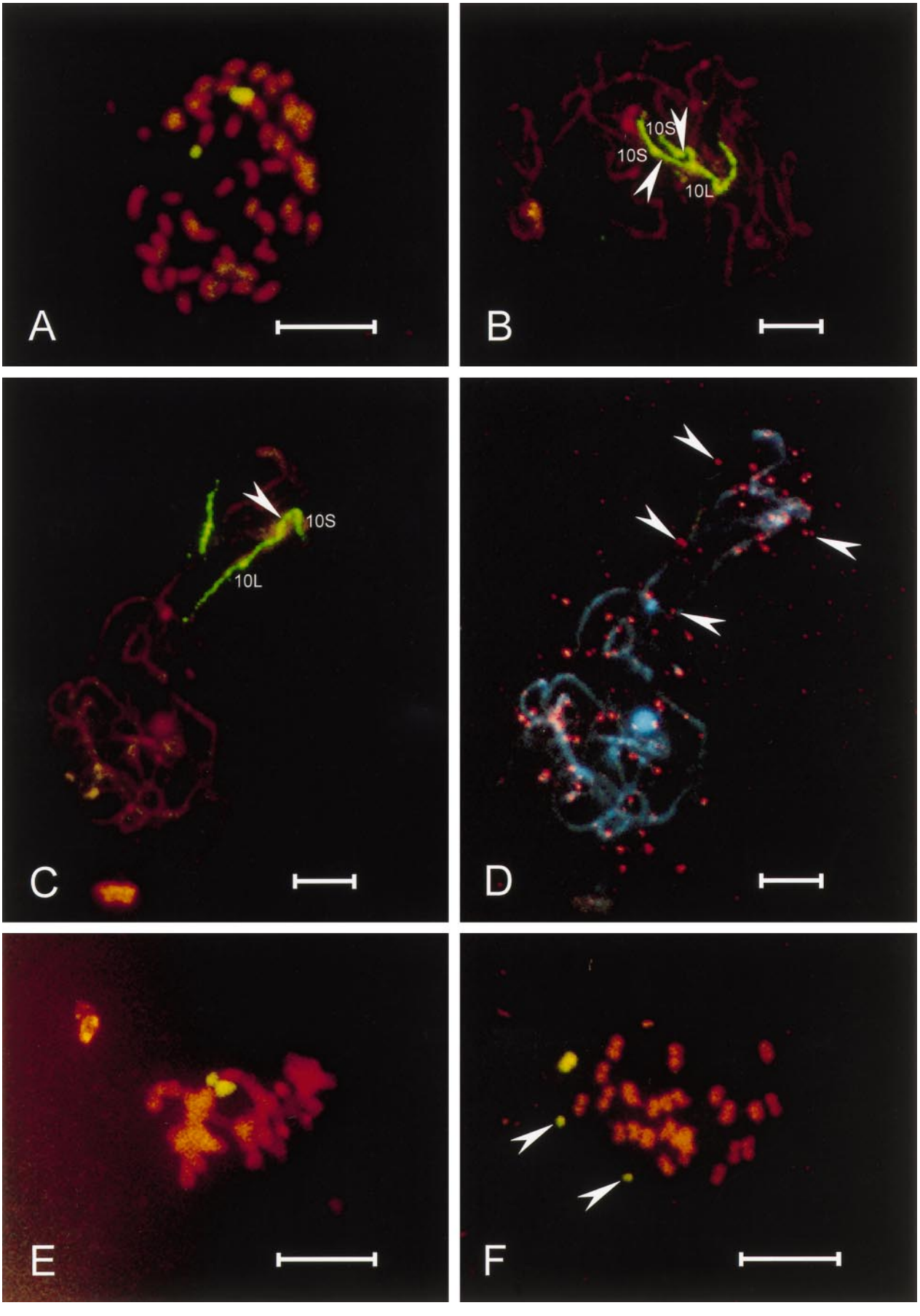
Alien chromosome distribution

In view of their pairing and chiasma formation, the two alien chromosomes were expected to disjoin more or less normally at anaphase-I (Fig. 2 A) and then divide and distribute regularly to the four poles at anaphase-II (Fig. 2 C). Although such regular behaviour of the bivalent and half-bivalents was observed in some of the pollen mother cells, deviations were seen in many cases. These deviations consisted of a precocious disjunction of bivalents at metaphase-I followed by the equational division of one or both half-bivalents at metaphase- or anaphase-I (Figs. 1 F and 2 B), and the lagging of half-bivalents as well as the irregular distribution of chromatids at anaphase-II (Fig. 2 D). Because the irregular distribution of half-bivalents and chromatids during meiosis determines the composition of the meiotic products, a quantitative estimate was made of the distribution of the large and small chromosomes during both meiotic divisions in pollen mother cells (Table 1). It was evident that, in more than 60% of the pollen mother cells of both first and second divisions, 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. 2 B), which apparently led to an irregular segregation to telophase-II nuclei in 27.5% of the cases (Table 1, Fig. 2 D). 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. 2 A and C).

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 was scored and the results are given in Table 2. A notable feature was that, despite its 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).

Female transmission of alien chromosomes

Unlike microsporogenesis, the cytological distribution of the alien chromosomes during megasporogenesis could not be determined because of technical difficulties in analysing meiosis in the ovules. However, it was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possible to determine the frequencies of transmission of both types of alien chromosomes from the BC_2 parent to BC_3 progeny through RFLP and GISH analyses. From the RFLP analysis of BC_3 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 at 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, which represented 23.9% of the total transmission. All these disomics consisted of one large and one 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 BC_3 population in which the presence of the alien chromosome was not detected through RFLP were also subjected to GISH analysis. Consistently, no alien chromosome was detected in any of them. The strict correspondence of the results obtained through both techniques confirmed that no indications of chimerism were found within these genotypes. In addition, from the observations through GISH analysis there was no evidence for the occurrence of homoeologuous recombination or for 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 was possible to identify the alien chromosomes and to pinpoint chromosome aberrations, a deletion in the present case, in BC_2 and BC_3 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 simply by genetic analysis. Furthermore, without cytological observations through GISH and FISH analyses, the detection of the interstitial deletion in one of the homologues would certainly have been impossible. Thus, a combination of RFLP analysis together with in situ DNA hybridisation techniques facilitates 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 by traditional cytogenetics in the past (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 when they occur in an alien background.

Fig. 1A–F Chromosome constitution and meiotic behaviour of an alien chromosome pair in the BC_2 clone 2101-1. Except for **D** the alien tomato chromosomes fluoresce yellow due to FITC labelling of tomato genomic DNA, whereas the potato chromosomes fluoresce orange-red due to the propidium iodide counterstain. Scale bars represent 10 μm. **A** Somatic chromosomes of 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 the centromere) and the other small. Note that the morphology of the large one conforms to chromosome-10 of tomato which is submedian. The smaller chromosome, because of its fold-back pairing, has a different morphology in this cell. **D** Same cell as in **C** probed with the telomeric repeat, pAtT4, using 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*

Occurrence and meiotic behaviour of the disomic addition

The occurrence of disomic additions of alien chromosomes in the backcross progeny is a particular 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 major hurdle for producing disomic additions from some of the monosomic additions that we have already selected (Garriga-Calderé et al. 1998). In all these cases, there is a general tendency for the alien chromosomes to behave abnormally during meiosis (Jacobsen et al. 1995; Garriga-Calderé et al. 1997, 1998) and, with certain frequencies they are expected to generate disomic additions. 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.

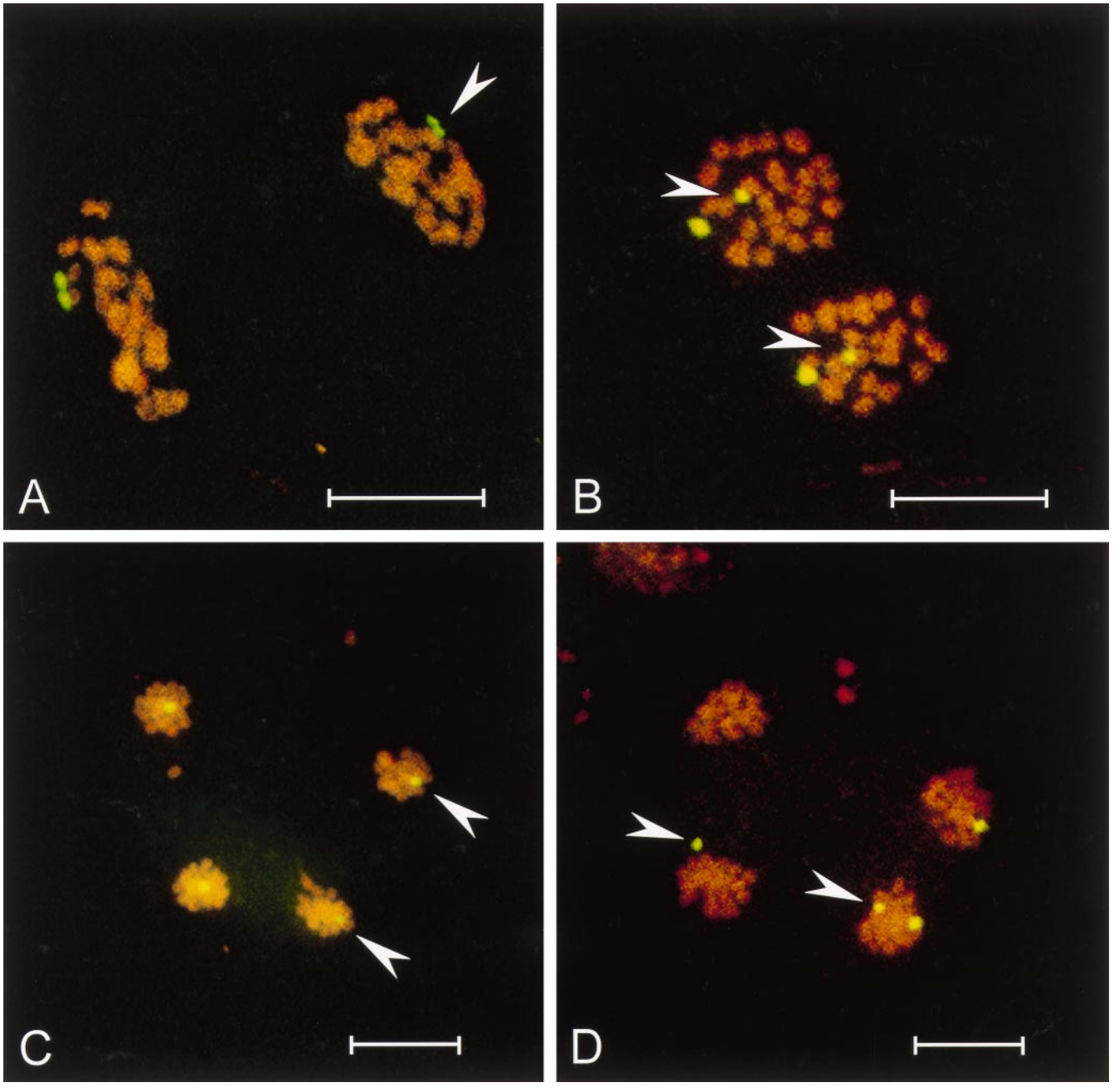

Fig. 2A–D Disjunction and division of alien chromosomes during microsporogenesis in the BC_2 clone 2101-1. **A–D** The alien tomato chromosomes fluoresce yellow due to FITC labelling of tomato genomic DNA, whereas the potato chromosomes fluoresce orange-red due to the propidium iodide counterstain. Scale bars correspond to 10 μm. **A** Telophase-I stage showing a normal disjunction of large and small (*arrow heads*) alien chromosomes to two poles. **B** Prophase-II stage showing the equational division of both large and small chromosomes that had occurred during anaphase-I. Note that a large and small chromosome are present at each pole. **C** Normal distribution of the four chromatids of the 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 the alien chromosomes. One large and one small chromosome are included in one nucleus whereas the others are in different nuclei, leaving one devoid of any alien chromosome

Because it is an interstitial deletion, in which the telomeres, sub-telomeres and centromere are intact, its normal behaviour can be readily explained. However, the reduction of its size relative to the normal homologue 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 a deletion is confined largely to the heterochromatic segment, as is the case in the present study, then the chromosome may not suffer any disadvantage in terms of survival and transmission.

Table 1 Distribution of alien chromosomes (large and small) to the poles at the 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 the inclusion of two or more alien chromosomes in one and the same pole, as well as lagging ones
[b] Possessing a large and a small chromosome in one nucleus and the other two in each of the two other nuclei

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

Number and types of chromosomes included	Number of nuclei	% Total number of nuclei	% 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

Table 3 Female transmission of alien chromosomes from BC_2 to BC_3 progenies estimated in a BC_3 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 an analysis of 15 randomly chosen plants
[b] All consisted of one large and one small alien chromosome

Transmission of the alien chromosomes

The rate of female transmission of the alien chromosome-10 from a BC_2 parent in a disomic condition to BC_3 progeny was fairly high (27.0%). Nevertheless, in a 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 the disomic, it was not as much as one might 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 the present 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 the meta/anaphase-I stages lead to a 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 BC_1 to BC_2 progenies, it was observed that a 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 differences among the alien chromosomes themselves might play an additional role in determining the rate of transmission. Besides the 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 univalents can greatly affect the proper distribution of two chromatids to the poles during meiosis. In view the much lower 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 in 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 BC_2 to BC_3 progenies of the two alien chromosomes (large and small) appeared to be very different. When considering only those BC_3 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 difference 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 BC_3 progenies that possessed both chromosomes (23.9%).

All the seven disomics recovered in the present study proved to possess one large and one small chromosome, although other possibilities such as two large or two small chromosome additions were also expected to occur. From the types of meiotic products observed during microsporogenesis, it appears that the frequencies of these possibilities are also very low (Table 2). The almost exclusive occurrence of both a large and a small homologue in one and the same telophase-II nucleus demonstrates that not only may two chromatids of a single alien chromosome, or half bivalent, be responsible for the occurrence of a disomic (De Jong et al. 1995) but also that two different chromatids of a pair of homologues are far more likely to be involved in the origin of a disomic. The 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.

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