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Cytogenetic analysis of *Lycopersicon esculentum* (+) *Solanum etuberosum* somatic hybrids and their androgenetic regenerants

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Abstract The aim of the study was to characterize genomic relationships among cultivated tomato (*Lycopersicon esculentum* Mill.) (2n=2x=24) and diploid (2n=2x=24) non-tuberous wild *Solanum* species (*S. etuberosum* Lindl.). Using genomic *in situ* hybridization (GISH) of mitotic and meiotic chromosomes, we analyzed intergeneric somatic hybrids between tomato and *S. etuberosum*. Of the five somatic hybrids, two plants were amphidiploids (2n=4x=48) mostly forming intragenomic bivalents in their microsporocytes, with a very low frequency of multivalents involving the chromosomes of tomato and *S. etuberosum* (less than 0.2 per meiocyte). Tomato chromosomes showed preferential elimination during subsequent meiotic divisions of the amphidiploids. Transmission of the parental chromosomes into microspores was also evaluated by GISH analysis of androgenic plants produced by direct embryogenesis from the amphidiploid somatic hybrids. Of the four androgenic regenerants, three were diploids (2n=2x=24 or 2n=2x+1=25) derived from reduced male gametes of the somatic hybrids, and one plant was a hypertetraploid (2n=4x+4=52). GISH revealed that each anther-derived plant had a unique chromosome composition. The prospects for introgression of desirable traits from *S. etuberosum* into the gene pool of cultivated tomato are discussed.

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Introduction

Introgression of desirable traits such as resistance to biotic and abiotic stresses into cultivated tomato (*Lycopersicon esculentum* Mill.) from wild species of the taxonomically close genus, *Solanum*, could be a valuable approach for tomato improvement, but intergeneric incompatibility barriers set a limit to the transfer of alien genes. Even though somatic hybridization has enabled combining the genomes of tomato and various *Solanum* species (Melchers et al. 1978; O'Connell and Hanson 1986; Jacobsen et al. 1992; Gavrilenko et al. 1992, 1994; Kobayashi et al. 1996), most of the symmetric and asymmetric intergeneric somatic hybrids obtained have been sterile, thereby preventing their backcrossing with tomato (Melchers et al. 1978; Derks et al. 1992; Lefrançois et al. 1993; Waara and Glimelius 1995). *Solanum etuberosum* Lindl. from the *Etuberosa* series, is one of the wild non-tuberous *Solanum* species with several desirable characters, such as tolerance to frosts (Hanneman and Bamberg 1986) and resistance to viral diseases (Harrison 1984; Thieme and Thieme 1998), but allotetraploid somatic hybrids between tomato and *S. etuberosum* could produce seedlings only through self pollinations (Gavrilenko et al. 1992). Therefore, the introduction of the desirable traits of *Solanum* spp. into tomato has been restricted even using somatic hybridization. However, intergeneric hexaploid *L. esculentum* (+) *S. tuberosum* somatic hybrids, for example, have resulted in a series of monosomic additions of tomato chromosomes in a cultivated potato (*S. tuberosum* L.) genetic background (Garriga-Calderé et al. 1998).

The main challenges for the successful introgression of genetic material from *Solanum* species into cultivated tomato have been: cytogenetic instability of the somatic hybrids (Wolters et al. 1994), preferential loss of tomato

chromosomes (Jacobsen et al. 1995; Garriga-Calderé et al. 1997), and a limited level of intergenomic chromosome pairing (Jacobsen et al. 1992; Gavrilenko et al. 1992). The polyploid genomic constitution of the somatic hybrids also limits their application in improving diploid (2n=2x=24) cultivated tomato. Tomato does not tolerate such genetic manipulation in contrast to the tetraploid (2n=4x=48) cultivated potato in which protoplast fusion has been successfully used for crop improvement (Waara and Glimelius 1995). One approach to reduce the ploidy level of the polyploid somatic hybrids is the production of haploid lines through anther culture (Rokka et al. 1995), but *in vitro* androgenesis of the tomato genome has been limited due to the lack of an efficient anther culture method (Gulshan et al. 1981; Summers 1997).

The objective of the study reported here was to determine by genomic *in situ* hybridization (GISH), the relationships between the genomes of tomato (2n=2x=24, herein marked by LL) and *S. etuberosum* (2n=2x=24, EE according to Ramanna and Hermsen 1981) in intergeneric symmetric somatic hybrids. Furthermore, reduction of the polyploidy of the somatic hybrids to form haploid lines for genome relationship analyses and tomato improvement is also described. In this article the term amphidiploid has been used for allotetraploid somatic hybrids possessing the diploid chromosome complements of both the parental species (LLEE); the term amphihaploid has been used for diploid (LE) androgenic regenerants derived from reduced gametes from amphidiploid hybrids (LLEE).

Materials and methods

Plant material

Five intergeneric somatic hybrids between *Lycopersicon esculentum* cv. Tamina and *Solanum etuberosum* k-9141 (N.I.Vavilov Institute of Plant Industry, Russia) produced previously (Gavrilenko et al. 1992, 1994) (Table 1) were included in this study.

Genomic *in situ* hybridization analysis

In total, 10–20 well-spread mitotic metaphase cells derived from root tips and 30–50 pollen mother cells (PMCs) of each hybrid genotype were analyzed. Chromosome spreads were prepared according to Zhong et al. (1996). To identify the parental chromosomes in the hybrids, we sonicated DNA from tomato (or occasionally DNA from *S. etuberosum*) (probe DNA) until the fragments attained the size of 1–5 kb and then direct labeled these with FITC–12-dUTP using a nick translation mix (Boehringer Mannheim). Blocking DNA (*S. etuberosum* or *vice versa*) was obtained by autoclaving total genomic DNA for 5 min, yielding fragments 100–500 bp in size. *In situ* hybridization was performed according to Schwarzacher and Heslop-Harrison (1994) with the modifications described by Kuipers et al. (1997). The hybridization mixture contained 50% deionized formamide, 10% (w/v) sodium dextran sulphate, 0.25% (w/v) SDS in 2× SSC, 1.5–2.0 ng/µl probe DNA, and 0.11–0.15 µg/µl blocking DNA. Chromosome preparations were counterstained with 2 µg/ml DAPI and 1 µg/ml propidium iodide and mounted in Vectashield. Slides were observed with an Olympus BX 60 microscope using the appropriate filters for FITC and DAPI. Digital images were recorded using a color CCD camera (Sony DXC-950P, Power Had) and analyzed with Soft Imaging System.

Table 1 Chromosome constitutions analyzed by GISH and DNA contents analyzed by flow cytometry in five somatic hybrids between *Lycopersicon esculentum* and *Solanum etuberosum*, and in androgenic regenerants of two hybrids (15.5.b and 6.a.19) (n.d. not determined)

Genotype	2n/Chromosome no.	Genomic composition	DNA content (pg)	Chromosome constitution of the hybrids. Number of chromosomes of:	
				L. esculentum	*S. etuberosum*
Parental lines:					
L. esculentum	2x/24	LL[b]	1.78	24	
S. etuberosum	2x/24	EE	1.62		24
Somatic hybrids:					
15.5.b	4x/48	LLEE	3.23	24	24
6.a.19	4x/48	LLEE	3.20	24	24
5.5.b	4x-1/47	LLEE	n.d.	24	23
5.17.a	4x+1/49	LLEE	n.d.	24	25
15.29.a	6x+1/73	LLEEEE	4.53	25	48
Androgenic regenerants:					
15.5.b.1.1.1	2x+1/25	LE	1.69	12	13
15.5.b.5.1.1.1[a]	2x/24	LE	1.74	12	12
15.5.b.5.1.1.2[a]	2x/n.d.		1.73	n.d.	n.d.
15.5.b.5.1.1.3[a]	2x/n.d.		1.68	n.d.	n.d.
15.5.b.5.2.1	4x+4/52	LLEE	3.07	26	26
6.a.19.5.1.1	2x+1/25	LE	n.d.	13	12

[a] Shoots derived from the same anther
[b] LL, Genome of tomato, *L. esculentum*; EE, genome of *S. etuberosum*

Production of haploids from intergeneric somatic hybrids

Pollination with S. phureja *(gynogenesis)*

Flower buds of greenhouse-grown tetraploid hybrids (15.5.b and 6.a.19) were emasculated and pollinated with a pollen mixture of two *S. phureja* clones (IVP48 and Norma). Immature seeds derived from berries at the age of 30–40 days were isolated and further cultivated on HLH medium (Neal and Topoleski 1983).

In vitro *androgenesis*

Anther culture was applied in two tetraploid somatic hybrids (15.5.b and 6.a.19) grown in a greenhouse where the temperature had been set as 23°C (day) and 18°C (night). Anthers were isolated from 3- to 5-mm-long flower buds and transferred to in vitro. Anther culture and shoot regeneration followed the protocol of Rokka et al. (1998). The number of embryos developed from anthers was counted after 4 weeks of culture, and the final number of shoots regenerated was analyzed after 4 months of culture. The shoots that successfully regenerated were rooted, and metaphase chromosome spreads of root tip meristems were prepared and analyzed by GISH as previously explained.

DNA content analyses

Flow cytometry was applied for the plant nuclear DNA content determination using a FACSort Becton Dickinson flow cytometer. Each plant (parental lines, hybrids, and anther-derived regenerants) was analyzed three times, with each sample containing chicken red blood cells (CRBC) as an internal DNA standard (2.33 pg of DNA; Galbraith et al. 1983) as previously described by Rokka et al. (1998).

Results

Chromosome composition of the intergeneric somatic hybrids

FITC-labeled genomic DNA of *S. etuberosum* blocked with unlabeled tomato genomic DNA (and *vice versa*) was hybridized *in situ* to mitotic metaphase chromosomes of the somatic hybrids. The hybridization of parental DNA was species-specific, with only satellite chromosomes no. 2 cross-hybridizing with the total genomic DNA of the parental species (marked with arrows, Fig. 1a), indicating highly conserved sequences in nucleolus organizer regions (NOR). Two hybrids (15.5.b and 6.a.19) of the five hybrids analyzed had the expected chromosome constitution containing the complete chromosome number of the both parents, i.e., 24 chromosomes of *S. etuberosum* and 24 chromosomes of tomato (Fig. 1a, Table 1) as determined by GISH. Two hybrids (5.5.b and 5.17.a) were hypo- and hypertetraploids having either a loss or a gain of one chromosome of *S. etuberosum*, but a full complement of tomato. One hybrid (15.a.29) was a hyperhexaploid with one extra chromosome of tomato.

Meiotic analysis of the somatic hybrids

Two tetraploid somatic hybrids (15.5.b and 6.a.19) with the expected chromosome numbers of 2n=48 were selected for meiotic analysis. Meiotic studies revealed 24 intragenomic bivalents at the diakinesis stage (Fig. 1c). Thus, all 12 chromosomes of both parental species were in pairs. This indicated that the hybrids 15.5.b and 6.a.19 were amphidiploids with the genomic constitution of LLEE. The meiotic chromosomal behavior of the intergeneric amphidiploids was characterized from the pachytene stage to the tetrad stage (Fig. 1b–h). In both of the amphidiploids, parental chromosomes paired autosyndetically, and the number of intragenomic bivalents ranged from 20 to 24 per cell at diakinesis (Table 2). Intergenomic bivalents were extremely rare (Fig. 1e); one homoeologous bivalent per meiocyte was found in only 2 PMCs of the 100 analyzed. Trivalent and quadrivalent formation, involving the chromosomes of tomato and chromosomes of *S. etuberosum*, was very seldom – less than 0.2 multivalents per PMC at diakinesis (Fig. 1d, Table 2).

At the pachytene stage, unpaired chromosomes were not observed (Fig. 1b), whereas at diakinesis from two to eight univalents per cell were revealed in more than 30% of the PMCs analyzed. At Metaphase I (M I), the frequency of the univalents had increased to about twice that observed at diakinesis (Table 2). This indicated precocious separation of the bivalents. GISH analysis revealed that at both the diakinesis and at the M I stages it

Table 2 Mean chromosome pairing per pollen mother cell (PMC) in the amphidiploid somatic hybrids at diakinesis and at metaphase I analyzed by GISH

Somatic hybrids	Number of PMCs scored (cells with univalents, %)	Frequency and type of chromosomal associations (min–max) per PMC					
		Mean number of univalents per PMC			Bivalents	Trivalents	Quadrivalents
		Total	*L. esculentum*	*S. etuberosum*			
at diakinesis							
15.5.b	30 (33%)	0.47 (0–4)	0.47 (0–4)	0	23.53 (22–24)	0.08 (0–1)	0.08 (0–1)
6.a.19	17 (42%)	1.02 (0–8)	0.84 (0–6)	0.18 (0–2)	23.00 (20–24)	0.06 (0–1)	0.06 (0–1)
at metaphase I							
15.5.b	26 (38%)	1.31 (0–6)	1.00 (0–4)	0.31 (0–2)	22.70 (20–24)	0.05 (0–1)	0
6.a.19	30 (70%)	2.10 (0–11)	1.70 (0–8)	0.40 (0–4)	21.90 (18–24)	0	0

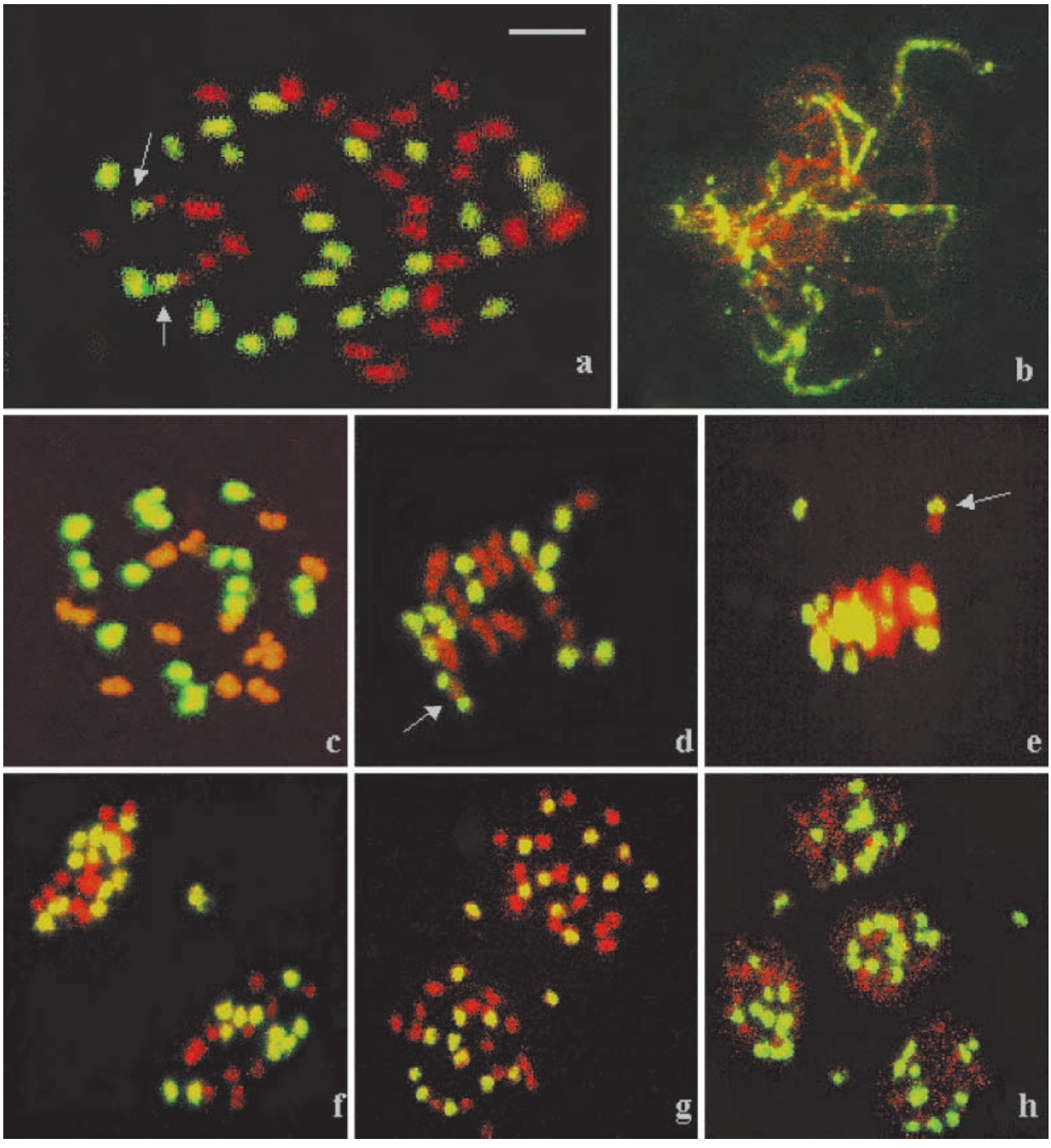

Fig. 1a Chromosome complement of the intergeneric amphidiploid 15.5.b (2n=4x=48, 24 chromosomes of tomato and 24 chromosomes of *S. etuberosum*). Somatic chromosomes of *S. etuberosum* fluoresce *yellow* due to the FITC-labeled *S. etuberosum* genomic DNA, whereas tomato chromosomes fluoresce *red* due to the propidium iodide counterstain. *Arrows* indicate the two satellite chromosomes of *S. etuberosum* with cross-hybridization in NOR regions. **b**–**h** Meiosis in intergeneric tomato (+) *S. etuberosum* amphidiploids (2n=4x=48, 24 tomato chromosomes and 24 chromosomes of *S. etuberosum*). In all the meiotic preparations, tomato chromosomes fluoresce *yellow* due to the FITC-labeled tomato genomic DNA, whereas *S. etuberosum* chromosomes fluoresce *red* due to the propidium iodide counterstain. **b** Late-pachytene stage showing a complete homologous pairing of tomato chromosomes, **c** preferential chromosome pairing within LL and within EE genomes at diakinesis – 12 bivalents of tomato and 12 bivalents of *S. etuberosum*, **d** metaphase I stage with putative trivalent (*arrow*), **e** metaphase I stage with association of tomato and *S. etuberosum* chromosomes (*arrow*), **f** anaphase I stage showing one laggard of tomato, **g** prophase II stage showing two laggards of tomato, **h** three tomato chromatids not incorporated in daughter nuclei at the telophase II stage. *Bar*: 10 μm

Table 3 Mean number and type of abnormalities during microsporogenesis in the amphidiploid somatic hybrids (15.5.b and 6.a.19) analyzed by GISH

Hybrid	Number of PMCs scored (% of cells with abnormalities)	Mean number (min–max) of laggards at anaphase I per PMC		
		Total	*L. esculentum*	*S. etuberosum*
15.5.b	40 (35.7%)	1.93 (0–4)	1.42 (0–4)	0.51 (0–3)
6.a.19	29 (55.1%)	2.21 (0–6)	1.78 (0–4)	0.43 (0–2)
		Mean number (min–max) of chromatids not incorporated in telophase II nuclei per PMC		
		Total	*L. esculentum*	*S. etuberosum*
15.5.b	48 (37.5%)	3.00 (0–8)	2.17 (0–6)	0.83 (0–4)
6.a.19	23 (64.3%)	4.80 (0–8)	3.60 (0–6)	1.20 (0–4)

Table 4 Production of androgenic lines through anther culture of two amphidiploid intergeneric somatic hybrids

Hybrid genotype cultured	Number of anthers plated	Number of anthers with embryo formation (%)	Total no. of shoots regenerated	Plants regenerated
15.5.b	119	4 (3.4%)	5	15.5.b.1.1.1 15.5.b.5.1.1.1[a] 15.5.b.5.1.1.2[a] 15.5.b.5.1.1.3[a] 15.5.b.5.2.1
6.a.19	245	3 (1.2%)	1	6.a.19.5.1.1

[a] Shoots derived from the same anther

was mainly the tomato bivalents that had undergone desynapsis: univalents were represented mostly by disjoined tomato chromosomes (Table 2). As a result of this, predominantly tomato chromosomes were revealed as laggards at the Anaphase I (AI), Telophase I (TI), and Prophase II stages (Fig. 1f,g, Table 3). On the basis of the chi square test, the frequencies of tomato univalents and tomato laggards were significantly ($P<0.01$) higher than those observed for *S. etuberosum* chromosomes. At Telophase II, it was mainly tomato chromatids that were not involved in microspore formation (Fig. 1h, Table 3).

Among the amphidiploids there was a distinct variation both in the frequency of the various meiotic irregularities analyzed and in the amount of pairing (Tables 2, 3), indicating a more regular meiosis in the hybrid 15.5.b than in the hybrid 6.a.19.

Production of haploid lines

Haploidization using gynogenesis by *S. phureja* pollination combined with immature seed culture resulted in no plantlets. Fourteen pistils of the hybrid 6.a.19 were pollinated, but only one parthenocarpic berry was developed. A total of 120 pistils of hybrid 15.5.b were pollinated, and 14 berries were formed, out of which eight immature seeds were found. However, the immature seeds cultivated *in vitro* showed no further development on HLH medium. During *in vitro* androgenesis, one anther of hybrid 15.5.b formed embryo structures on induction medium after the first 4 weeks of culture (Fig. 2a). After a subsequent 8 weeks of culture, a total of 4 (3.4%) out of 119 anthers plated from hybrid 15.5.b produced embryos (Table 4). After a total of 4 months of culture, five shoots were regenerated from hybrid 15.5.b, of which three were derived from separate anthers. Similarly, from hybrid 6.a.19, 3 (1.2%) out of 245 anthers produced embryos (Table 4), but only one green shoot was regenerated directly on induction medium. The remaining anther-derived embryos failed to form green shoots (Table 4). Of the haploids transferred to the greenhouse, one plant (16.a.19.5.1.1) died, but the four haploids derived from amphidiploid somatic hybrid 15.5.b. grew vigorously *in vivo* in the greenhouse (Fig. 2f).

Chromosome composition and DNA content of the androgenic regenerants

Four androgenic plants, each derived from a separate anther of the amphidiploid somatic hybrids (15.5.b and 6.a.19), were analyzed by GISH for their mitotic chromosome composition. Three plants were found to contain the diploid level (2n=2x), but two of these were aneuploids containing 25 chromosomes (Table 1). The aneuploid 15.5.b.1.1.1 had the constitution of 12 tomato

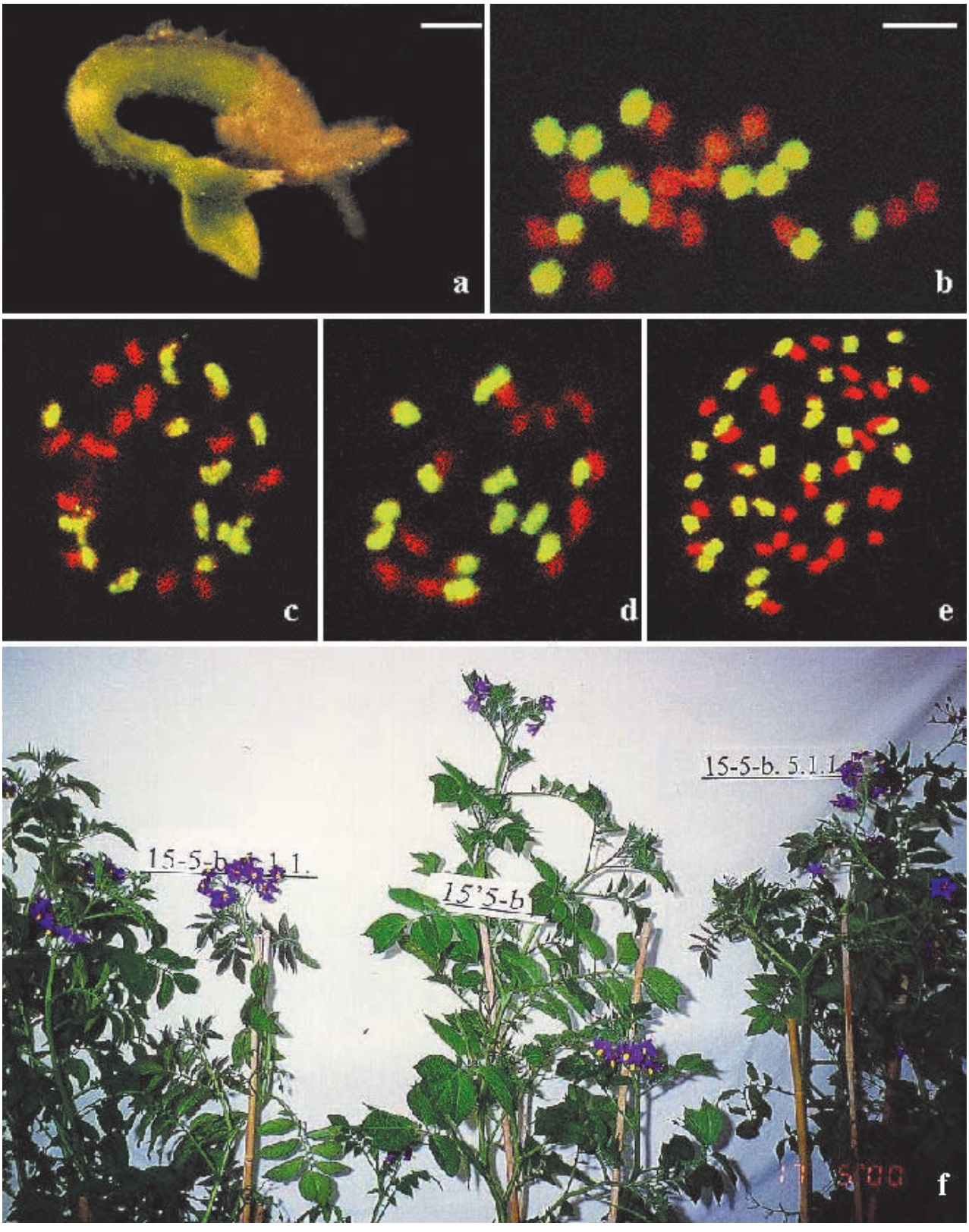

Fig. 2 **a** Shoot regeneration from an anther of a tetraploid *L. esculentum* (+) *S. etuberosum* intergeneric somatic hybrid. *Bar*: 0.6 mm. **b**–**e** Chromosome complements of anther-derived regenerants. Tomato chromosomes fluoresce *yellow* due to the FITC labeling and *S. etuberosum* chromosomes fluoresce *red* due to the propidium iodide counterstain; *bar*: 10 µm. Somatic chromosomes of: **b** regenerant 15.5.b.1.1.1 (2n=2x+1=25: 12 chromosomes of tomato and 13 chromosomes of *S. etuberosum*), **c** regenerant 6.a.19.5.1.1 (2n=2x+1=25: 13 chromosomes of tomato and 12 chromosomes of *S. etuberosum*), **d** regenerant 15.5.b.5.1.1 (2n=2x=24: 12 chromosomes of tomato and 12 chromosomes of *S. etuberosum*), **e** regenerant 15.5.b.5.2.1 (2n=4x=52: 26 chromosomes of tomato and 26 chromosomes of *S. etuberosum*). **f** Plant morphology of anther-derived amphihaploid (2n=2x+1=25) 15.5.b.1.1.1 (*left*), amphidiploid (2n=4x=48) intergeneric somatic hybrid (15.5.b.) between tomato and *S. etuberosum* (*middle*), and anther-derived amphihaploid (2n=2x=24) 15.5.b.5.1.1 (*right*) in the greenhouse

chromosomes and 13 *S. etuberosum* chromosomes (Fig. 2b). On the contrary, the aneuploid 6.a.19.5.1.1 had 13 tomato chromosomes and 12 *S. etuberosum* chromosomes (Fig. 2c). One plant (15.5.b.5.1.1.1) was euhaploid (2n=2x=24) containing 12 chromosomes of tomato and 12 chromosomes of *S. etuberosum* (Fig. 2d). The remaining anther-derived plant (15.5.b.5.2.1) was a hypertetraploid (2n=4x+4=52) with a genomic constitution of 26 tomato chromosomes and 26 *S. etuberosum* chromosomes (Fig. 2e, Table 1). The flow cytometric analyses supported the results obtained by GISH. The 2 C values of the diploid androgenic plants were close to the 2 C values of the diploid parental species (Table 1).

Discussion

The present study showed that using GISH, the chromosomes of *L. esculentum* (L genome) and *S. etuberosum* (E genome) which are similar in morphology, can be discriminated in hybrid nuclei for an analysis of intergenomic relationships. Furthermore, the transmission potential of tomato chromosomes and *S. etuberosum* chromosomes through meiotic divisions of intergeneric somatic hybrids was determined by GISH analysis of androgenic haploids. Haploid lines were produced through direct embryogenesis using *in vitro* androgenesis. This is the first reported success of direct embryogenesis in anther culture of tomato material.

In somatic hybrids between two genetically distant species, the species-specific elimination of somatic chromosomes is frequent (Pental et al. 1986; Babiychuk et al. 1992; Garriga-Calderé et al. 1997), but our tomato (+) *S. etuberosum* hybrids were relatively stable in terms of their somatic chromosome complements. Of the five hybrids analyzed, two had intact parental genomes, and three hybrids had lost or gained only one chromosome of either tomato or *S. etuberosum*. Quite similar observations in chromosome elimination were also previously described in interspecific tuber-bearing potato (+) *S. etuberosum* somatic hybrids (Dong et al. 1999).

Despite the relative mitotic stability of the tomato (+) *S. etuberosum* hybrids seen in our study, we did observe a preferential loss of tomato chromosomes during meiosis of the intergeneric amphidiploids (LLEE) analyzed. Our data are concordant with results from recent investigations on tomato (+) *S. tuberosum* hybrids (Jacobsen et al. 1995; Garriga-Calderé et al. 1997), in which low transmission rates of particular tomato chromosomes to gametes were detected. Preferential elimination of the chromosomes of tomato might be due to an asynchrony in the meiotic cycles between L- and of E- parental genomes in hybrid nuclei leading to a precocious separation of the tomato bivalents. Another explanation might be due to the peculiarities of genome interactions, resulting in a disruption of the pairing regulatory mechanism and in a desynapsis of tomato bivalents as observed in sexual intergeneric *L. esculentum*×*S. lycopersicoides* hybrids (Menzel 1962).

The degree of homoeologous chromosome pairing is essential for an assessment of the potential for introgression of alien genes. For example, diploid sexual hybrids between tomato and its taxonomically closest relative in the genus *Solanum* (*S. lycopersicoides*) showed quite frequent homoeologous pairing (Menzel 1962), and gene transfer was achieved through meiotic crossing-over in backcross progenies (De Verna et al. 1987). In contrast, in the tomato and cultivated potato combination which is phylogenetically more distant, a very low frequency of allosyndetic pairing was observed (Garriga-Calderé et al. 1999). The introgression of alien genetic material was achieved through interspecific translocation (Garriga-Calderé et al. 1997) and through establishing monosomic addition lines (Garriga-Calderé et al. 1998). Our GISH results suggest that intergeneric hybrids mainly showed preferential bivalent pairing within the EE and within the LL genomes and, therefore, the potential for intergenomic recombination through meiotic crossing-over is very low. The mostly autosyndetic pairing in intergeneric hybrids may be due to a low degree of linear homology between the L- and E-genomes caused by intragenomic structural rearrangements. Evidence of this was reported by Perez et al. (1999) when the genetic map of the E genome showed conservation of most of the linkage groups with the L genome of tomato and A genome of cultivated potato, but various translocations and possible inversions and transpositions were detected among the genomes. The low level of intergenomic pairing in our amphidiploids (LLEE) requires tools to enhance homoeologous chromosome pairing in order to achieve intergeneric recombination. Chromosome exchanges between parental genomes could be promoted by using irradiation (Friebe et al. 1994) or through *in vitro* culture of the hybrids (Wolters et al. 1994; Garriga-Calderé et al. 1997). Moreover, reducing the ploidy level of the amphidiploid somatic hybrids may enhance the degree of intergenomic pairing because amphihaploids have only one set of the L- and the E-genomes and, consequently, have no option for homologous pairing. In the present study, to reduce the ploidy level and enhance homoeologous chromosome pairing of the tetraploid somatic hybrids, we engineered haploidization of the hybrid genome by using (1) gynogenesis with *S. phureja* pollination that generally leads to haploid formation in potato, and (2) *in vitro* androgenesis. Whereas the first approach was completely unsuccessful in our intergeneric hybrids, the second method did lead to the production of the haploid lines. In earlier studies, tomato has been recalcitrant in anther culture (Gresshoff and Doy 1972; Summers 1997; Zagorska et al. 1998), and attempts on direct embryogenesis have been completely unsuccessful (Cappadocia and Sree Ramulu 1980; Summers 1997). In our study, tomato (+) *S. etuberosum* hybrids, however, had androgenic capacity. To our knowledge, this is the first report of direct embryogenesis in anther culture for tomatoes. Of the four androgenic regenerants analyzed by GISH, three were amphihaploids at the diploid level. The amphihaploids were either eudiploid (2n=2x=24) or hyperdiploid

(2n=2x+1=25). Regenerants with a hypodiploid chromosome number (less than 24 chromosomes) were not detected, possibly indicating that the gamete viability of the intergeneric hybrids was dependent on the presence of the complete parental genomes (12 tomato chromosomes and 12 *S. etuberosum* chromosomes). One plant regenerated from an anther of somatic hybrid 15.5.b was a hypertetraploid with a chromosome number of 52. Based on the microsporogenesis analyses, the hybrid donor plants did not produce unreduced gametes. Most probably, the hypertetraploid regenerant was derived from an aneuploid microspore (13 tomato chromosomes and 13 *S. etuberosum* chromosomes), and it had followed somatic doubling during *in vitro* culture. In the greenhouse, most of the anther-derived regenerants grew very vigorously.

The successful production of androgenic regenerants may present new perspectives in the hybridization of tomato with its distant relatives in the future. For example, through *in vitro* androgenesis of allohexaploid somatic hybrids (LLLLSS) it is possible to obtain sesquidiploid genotypes (LLS) that could be used for establishing alien addition lines in the tomato background, as has already been achieved in the tomato× *S. lycopersicoides* hybrids (De Verna et al. 1987; Chetelat et al. 1998).

Haploid lines derived from our intergeneric somatic hybrids might also help to accelerate genome analysis. Controlling intergenomic pairing may become less stringent in amphihaploids which do not offer conditions for preferential pairing due to the absence of homologs. The potential of intergenomic pairing could be more completely measured by analyzing the amphihaploid lines in future. Further research is also needed to focus on the assessment of recombination between homoeologous chromosomes of E- and L- genomes at the diploid level.

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