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RFLP analysis and genomic in situ hybridization (GISH) in somatic hybrids and their progeny between *Lycopersicon esculentum* and *Solanum lycopersicoides*

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Abstract RFLP (restriction fragment length polymorphism) and GISH (genomic in situ hybridization) analyses were employed to identify the chloroplast and nuclear genomes of the somatic hybrids and progeny between tomato 'Ohgata zuiko' and *Solanum lycopersicoides* ('LA 2386'). A random distribution of the chloroplast genotype was determined using a cloned 19.6-kb *Bam*HI fragment (Ba1) of tobacco chloroplast DNA. Eight selected hybrids were analyzed for their chromosomal compositions; 4 were tetraploids (2n = 48) with an equal number of chromosomes derived from each parent as accurately determined by GISH, and the other 4 were hexaploids, containing an average of two sets of tomato chromosomes and one set from the wild parent. RFLP analysis with six tomato nuclear probes of known chromosomal locations revealed no major variation among the 44 hybrid plants surveyed. However, it also showed the presence of both parent-specific alleles and the loss of some and the presence of a few non-parental alleles, indicating rearrangement and/or recombination of the nuclear DNA. The relevance of the molecular and cytological methods and the potential use of somatic hybrids for plant breeding are demonstrated.

Key words Tomato and *Solanum lycopersicoides* intergeneric hybrid · Chloroplast DNA · Nuclear genome · RFLP · GISH

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Introduction

Wild nightshade, *Solanum lycopersicoides* endemic to the Southern Peru and Northern Chile, has an economic importance as the genetic source of several characters such as high tolerance to low temperature, resistance to disease caused by cucumber mosaic virus and *Botrytis* mold, a large group of monogenic characters and flowering sensitivity to long day (Rick and Yoder 1988). Although transmission of desired characters from this wild species to cultivated tomato has been carried out, *S. lycopersicoides* can only be crossed when tomato is used as the female parent, producing low yield but viable hybrid seeds (Rick 1951). Until recently, further progress has been prevented by the high sterility of the intergeneric hybrids. Chetelat et al. (1997) successfully obtained 280 BC_1 plants after direct backcrossing of a partially male-fertile F_1 sexual hybrid to tomato at the diploid level.

Somatic hybridization by chemically induced fusion of protoplasts between tomato and *S. lycopersicoides* was reported by Handley et al. (1986) as an alternative process to transmit useful traits from the latter into tomato. Low pollen fertility and limited fruit set in the hybrids were clearly observed. On the other hand, Hossain et al. (1994) developed a large number of both male-fertile and female-fertile somatic hybrids by electrofusion of mesophyll protoplasts of tomato and *S. lycopersicoides*. To date, 70 of these plants and their progeny have been maintained for over 3 years.

In order to fully understand the merit of somatic hybridization in the production of intergeneric hybrids as well as the utilization of the fertile hybrids produced, we have to characterize the hybrids.

Due to the relatively small-sized chromosomes of tomato and *S. lycopersicoides*, determination of the exact chromosomal composition within hybrids was

difficult. However, with the advance of a new technique known as genomic in situ hybridization (GISH), the parental origin of chromosomes of 8 of the mentioned hybrid plants could be precisely identified using genomic DNA of *S. lycopersicoides* as the probe. This technique has successfully been utilized to identify parental genomes in other intergeneric somatic hybrids (Wolters et al. 1994; Ramulu et al. 1996) and intergeneric sexual hybrids and hybrid derivatives (Schwarzacher et al. 1989; Thomas et al. 1994; D'Hont et al. 1995) and to study the genome organization and evolution of natural plant species of rather uncertain origin (Bennett et al. 1992; Chen and Armstrong 1994; Fukui et al. 1997, 1998). Moreover, we also analyzed the somatic hybrids and their progeny for its chloroplast (cp) DNA inheritance and nuclear DNA content using restriction fragment length polymorphism (RFLP) markers.

Materials and methods

Plant materials

Greenhouse-grown F_1 somatic hybrids and their progeny were used in the study. The F_1 somatic hybrids were obtained by electrofusion between mesophyll protoplasts of *Lycopersicon esculentum* 'Ohgata zuiko' and *Solanum lycopersicoides* ('LA 2386'). Details of their origin, in vitro culture, morphology, isozyme analysis, randomly amplified polymorphic DNA (RAPD) analysis and cytology have been described by Hossain et al. (1994).

RFLP

Total DNA was extracted from fresh leaf tissues of greenhouse-grown hybrids which had been maintained for over 3 years and from the parental species as described by Draper and Scott (1988). For the cp DNA analysis, 5 μg of total DNA was digested with restriction endonucleases *Hae*III, *Eco*RV, *Sac*I and *Xba*I. For analysis of the nuclear genome, 10–15 μg of total DNA was digested with *Bam*HI, *Hae*III, *Eco*RI, *Eco*RV, *Hin*dIII, and *Xba*I. DNA electrophoresis on 0.7% agarose gels, transfer of DNA to nylon filter membranes (Micro Separations, USA) by Vacu gene XL (Pharmacia, Sweden) and DNA hybridization and detection using a non-radioactive DIG detection kit (Boehringer Mannheim, Germany) were performed according to the manufacturers' instruction.

A plasmid, pTBa1 (Sugiura et al. 1986) with a 19.1-kb *Bam*HI insert (Ba1) of tobacco cpDNA was obtained from the Center for Gene Research, Nagoya University, Japan, and used as the probe for the cpDNA analysis. The tomato RFLP probes (Tanksley et al. 1992; Tanksley 1993) kindly provided by Prof. S. Tanksley, Cornell University, USA, used for the nuclear DNA analysis were as follows, CT106A (chromosome 2), TG132 (chromosome 3), TG609 (chromosome 4), TG590 (chromosome 6), TG434 (chromosome 8) and TG28A (chromosome 12). The inserts were excised from the vector with the appropriate restriction enzyme and separated from the vector on a 0.7% (w/v) agarose gel. The purified insert DNAs were recovered through SUPREX^{TM}01 (Takara, Japan). The insert DNAs were labeled with digoxigenin-11-dUTP (Boehringer) by a random primer labeling method as described by the manufacturer.

Genomic in situ hybridization (GISH)

Young shoot cuttings of the hybrids were collected and grown in water for 2–3 weeks to stimulate new root growth. Root tips (1–2 cm) were excised, pretreated in an aqueous solution of 2 m*M* 8-hydroxyquinoline for 2.5–3 h at 17°C, fixed in 3 : 1 ethanol : acetic acid for 1 h to 2 weeks and stored in 70% ethanol at 4°C. The fixed root tips were rinsed in distilled water for 20–30 min and incubated in an enzymatic mixture (2% Cellulase Onozuka RS (Yakult, Tokyo), 0.3% Pectolyase Y-23 (Seishin Pharmaceutical, Tokyo), 1.5% Macerozyme R-200 (Yakult, Tokyo), 1 m*M* EDTA, pH 4.2; Fukui et al. 1994) in 30 m*M* citrate buffer, pH 4.2 at 37°C for 30 min. The partially digested root tips were rinsed gently in distilled water and subsequently squashed on a clean glass slide in a drop of fresh 3 : 1 ethanol : acetic acid with a pair of forceps. Slides were stored at − 20°C for up to 6 months or used immediately. Good samples were treated with 100 μg/ml RNase A (Sigma) in 2 × SSC at 37°C for 30 min, washed in 2 × SSC, dehydrated through 70%, 95% and 99% ethanol series at room temperature for 5 min each and air dried.

In situ hybridization was performed following the method described by Ohmido and Fukui (1995) with slight modifications. The DNA of *Solanum lycopersicoides* was labeled using a biotin-high prime labeling kit (Boehringer) following the manufacturer's instruction. The tomato total DNA (used as the blocking DNA) was autoclaved for 5 min giving fragments of about 100 bp. The hybridization mixture (16 μl per slide) consisted of 50% formamide (Boehringer), 10% sodium dextran sulphate, 2 × SSC, 100 ng of biotinylated-*Solanum lycopersicoides* DNA and 3 μg of tomato DNA. The probe mixture was denatured for 10 min at 95°C, quickly placed in ice for 5 min and loaded onto the slides. The samples were covered with cover slips, redenatured for 2 min at 80°C and placed in a pre-warmed humid box at 37°C overnight. The slides were then washed consecutively with 2 × SSC, 50% formamide/2 × SSC, 2 × SSC again and 4 × SSC for 10 min each, at 40°C. The probe was detected with 20 ng/ml fluorescein isothiocyanate (FITC)-avidin conjugate (Boehringer) and amplified with 15 ng/ml biotinylated anti-avidin solution (Vector Laboratory, USA) and 20 ng/ml fluorescein-avidin solution (Vector Lab) at 37°C for 60 min each. Blocking was carried out three times before each immunological reaction using 5% bovine serum in the first and third blocking steps and goat serum albumin in the second blocking step, in BT buffer at 37°C for 5 min each. The slides were mounted with Vectashield (Vecta Laboratories) containing 1 μg/ml propidium iodide (PI).

Fluorescence microscopy and image analysis

Fluorescent images were observed by a fluorescence microscope (Axiophot, Zeiss) with B- and G-light excitation filters (B10, G15). Chromosome images were digitized by a cooled CCD camera (PXL 1400, Photometrics) and were subjected to image analyses as described by Ohmido and Fukui (1996).

Results

Chloroplast DNA analysis

The chloroplast genotypes of the somatic hybrids and some progeny were determined using a cloned 19.6-kb *Bam*HI fragment (Ba1) of tobacco cpDNA. Ba1 hybridized to *Hae*III-, *Eco*RV-, *Sac*I- and *Xba*I-digested DNA fragments. However, polymorphisms between the fusion parents were revealed only when the DNA was digested with *Sac*I (Fig. 1A) and *Hae*III (Fig. 1B).

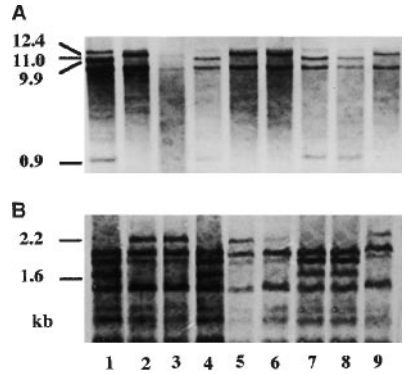

Fig. 1A, B Analysis of chloroplast DNA. Five μg of (**A**) *Sac*I- and (**B**) *Hae*III- digested total DNA of the tomato parent (*lane 1*), *S. lycopersicoides* parent (*lane 2*) and selected somatic hybrids (*lanes 3–9*), was hybridized with the tobacco cpDNA probe, Ba1. The sizes of species-specific fragments are indicated in kilobases

The cpDNA sequences detected were the 3 tomato-specific *Sac*I fragments of 12.4, 9.9 and 0.9 kb, the *S. lycopersicoides*-specific *Sac*I fragment of 11.0 kb, the tomato-specific *Hae*III fragment of 1.6 kb and the *S. lycopersicoides*-specific *Hae*III fragment of 2.2 kb. With the probe/enzyme combinations used, the parental species also had one or more fragments in common.

Southern analysis of the 33 hybrids digested with *Sac*I and *Hae*III and probed with Ba1 showed that all the RFLP patterns were identical to those of the parents. Representatives are shown in Fig. 1A and B. The hybrids contain only the tomato-specific fragment (lanes 4,7 and 8), only the *S. lycopersicoides*-specific fragment (lanes 3,5,6 and 9) or fragments from both fusion parents (not shown). Based on these species-specific RFLPs, a chloroplast genotype was assigned to each hybrid (Table 1). Sixteen hybrids contained cpDNA fragments specific to tomato, 16 had cpDNA fragments specific to *S. lycopersicoides* and 1 hybrid (C7S3) possessed cpDNA fragments specific to both parents. No new or non-parental fragment was found in this enzyme/probe combination. The results obtained with the Ba1/*Hae*III combination were consistent with those from the Ba1/*Sac*I combination except for the absence of *S. lycopersicoides* DNA in 1 plant (C7S3) that was identified in the Ba1/*Hae*III combination.

Genome analysis by GISH

Among the thriving individual somatic hybrid plants between tomato and *S. lycopersicoides*, there were distinct differences in leaf morphology and leaf color. The hybrid plants were then classified into two groups based on their leaf characteristics: (1) leaves were fairly light green and had smooth surface, (2) leaves were fairly dark, broader and had a rough surface. Preliminary cytological investigation showed that the hybrid plants belonging to the first group were tetraploids (2n = 48), while hybrid plants identified in the second group were hexaploids (2n = 72).

GISH was carried out to distinguish the exact chromosome sets derived from each parent in metaphase spreads of 4 tetraploid and 4 hexaploid somatic hybrid plants. Figure 2A and B show yellow-green fluorescent signals on 24 chromosomes which identify their origin to be *S. lycopersicoides*. The signals were consistently observed on the 24 larger chromosomes of all the hybrid plants analyzed. The fluorescent signals also covered almost the entire length (euchromatic region) of the chromosomes, except for a few chromosomes in 1 tetraploid and 1 hexaploid hybrid plant. Short unlabeled regions in some of the fluorescent chromosomes of *S. lycopersicoides* (Fig. 2C) and the interstitial fluorescent signals in some unlabeled tomato chromosomes were also observed (Fig. 2D). These plants were both female-fertile and male-fertile but produced neither fully developed seeds nor culturable embryos.

Telomeric ends as well as the two presumed satellite chromosomes (indicated by arrowheads in Fig. 2A and C) were characterized by stronger fluorescent signals, indicating the localization of repetitive sequences.

The remaining 24 and an average of the 48 smaller chromosomes of tomato origin in the tetraploid and hexaploid hybrid plants, respectively, showed little hybridization and exhibited red fluorescence upon counterstaining with PI.

Table 1 Chloroplast DNA type of somatic hybrids and progeny between *L esculentum* 'Ohgata zuiko' and *S. lycopersicoides* (LA 2386) (*TC L. esculentum* genotype · *SL S. lycopersicoides* genotype · *RC* recombinant (both parental genotypes) · *nd* not determined)

Plant accession	cpDNA type		Plant accession	cpDNA type	
	Restriction enzyme			Restriction enzyme	
	*Hae*III	*Sac*I		*Hae*III	*Sac*I
C78S8	SL	SL	C77S2	TC	TC
C69S5	SL	SL	C85S3	TC	nd
C11S5	TC	nd	C77S3	TC	TC
C48S3	TC	TC	C19S6	TC	TC
C15S4	TC	TC	C58S4	TC	TC
C18S5	SL	nd	C7S3	RC	TC
C22S2	TC	TC	C15S1	TC	TC
C81S7	SL	SL	C7S10 F2	TC	TC
C11S6	SL	SL	C14S2 F2	TC	TC
C18S1B	SL	nd	C81S4B F2	SL	SL
C87S8	SL	SL	C11S8 F2	SL	SL
C13S2	TC	nd	C18S3 F2	SL	SL
C81S3	SL	SL	C23S3 F3	SL	SL
C11S4	TC	TC	C15S2 F3	TC	TC
C11S6B	SL	SL	C18S6B F2	SL	SL
C69S3 F2	SL	SL	C24S8 F2	SL	SL
C23S8 F2	TC	TC			

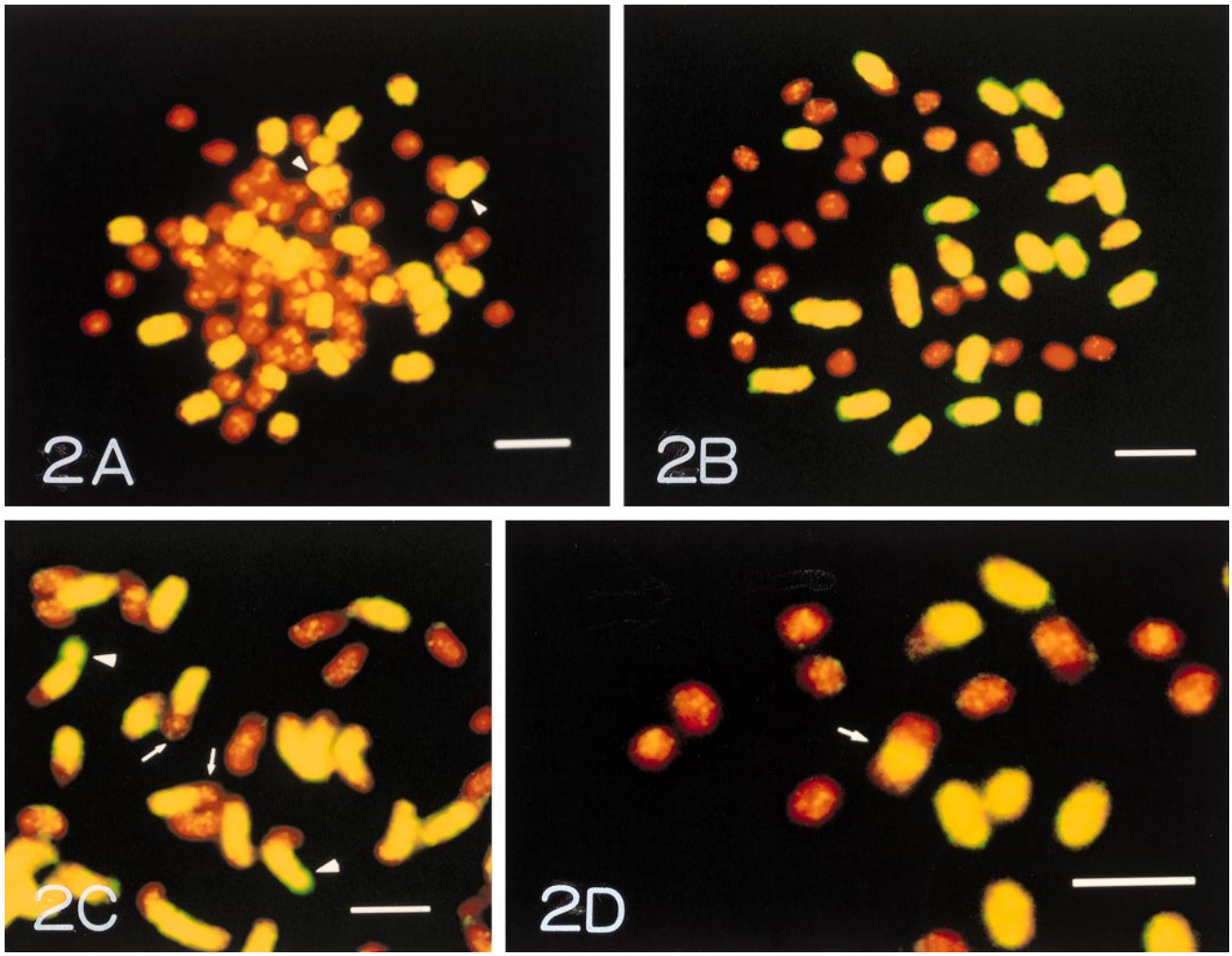

Fig. 2A–D GISH of hexaploid (**A**) and tetraploid (**B**) hybrid plants with 24 larger chromosomes showing hybridization with the probe (*yellow-green*). Tomato chromosomes are counterstained red with PI. Some unlabeled regions (*arrowed*) in *S. lycopersicoides* chromosomes (**C**) and the interstitial site of hybridization (*arrowed*) in tomato chromosome (**D**) are detected in some cells of some intergeneric hybrids. *Arrowheads* in **A** and **C** are the satellite chromosomes of *S. lycopersicoides*. *Bars*: 5 μm

Detection of RFLPs of the nuclear DNA by Southern hybridization analysis

Six tomato nuclear probes were hybridized to total DNAs of *L. esculentum* and *S. lycopersicoides* digested with six restriction enzymes. The probes showed polymorphism between the parents with at least two restriction enzymes (Table 2). RFLPs with multiple fragments were often produced in the different probe/enzyme combinations. Also, a limited number of fragments common to both parents were found.

Twelve probe/restriction enzyme combinations were selected to characterize the nuclear DNA of 11–44 hybrid plants. In all the probe/enzyme combinations, all of the hybrid plants showed similar hybridization patterns indicating that all of the plants analyzed were homozygous at the six loci examined. The numbers of common fragments, parent-specific fragments, new fragments and lost parent-specific fragments per probe/enzyme combination were counted in the hybrid plants. The total number of fragments counted in the 12 hybridizations by the different probe/enzyme combinations is listed in Table 3. With the six probes, 42 fragments were generated, 17 of which were specific to *L. esculentum*, 14 were specific to *S. lycopersicoides*, 6 were common fragments to both parents and 5 were new, non-parental fragments. In most probes, there was also a loss of at least 1 *S. lycopersicoides*-specific fragment, while there were limited losses from the tomato parent. Hybridization patterns to parental DNAs and DNAs of selected hybrids and their progeny are presented in Fig. 3 for *Eco*RV digestion. Hybridization of the CT106A probe to the total DNA of the hybrids digested with *Eco*RV produced only two RFLPs: (1) a pattern identical to the parents and (2) a new

Table 2 RFLPs between *L. esculentum* and *S. lycopersicoides* with nuclear probes (*nd* not determined)

Locus location	Probe	Restriction enzymes					
		*Bam*HI	*Hae*III	*Eco*RI	*Eco*RV	*Hin*dIII	*Xba*I
Chromosome 2	CT106A	+[a]	+	+	+	+	nd
Chromosome 3	TG132	±	nd	+	+	+	+
Chromosome 4	TG609	+	+	+	+	+	−
Chromosome 6	TG590	+	±	+	+	+	−
Chromosome 8	TG434	±	+	±	+	±	+
Chromosome 12	TG28A	±	±	+	+	+	−

[a] +, RFLP present; −, RFLP absent; ±, no useful RFLP (only one of the parents has one or more specific bands)

Table 3 RFLP analysis of the intergeneric somatic hybrids and their progeny using 12 probes and restriction enzyme combinations

Probes	Restriction enzymes	Number of specific fragments present[a]				Number of specific fragments absent	
		C	L	S	N	L	S
CT106A, Ch. 2	*Eco*RV, *Hin*dIII, *Hae*III	2	6	5	1	1	4
TG132, Ch. 3	*Hin*dIII, *Eco*RI, *Eco*RV	2	4	3	3	1	5
TG609, Ch.4	*Bam*HI	1	1	1	0	0	0
TG590, Ch.6	*Eco*RI, *Bam*HI, *Hin*dIII	0	5	4	0	0	1
TH434, Ch. 8	*Hae*III	0	1	1	0	0	0
TG28A, Ch.12	*Hin*dIII	1	0	0	1	0	1
Total		6	17	14	5	2	11

[a]C, Common; L, *L. esculentum*; S, *S. lycopersicoides*; N, new fragment

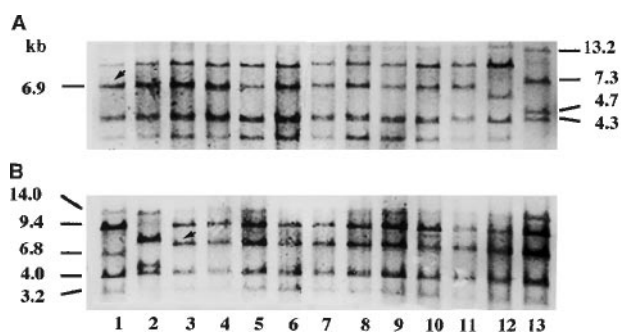

Fig. 3A, B Nuclear DNA analysis. Hybridization of CT 106A tomato genomic probe with *Eco*RV-digested DNA of the tomato parent (*lanes A12*, *B1*), *S. lycopersicoides* parent (*lanes A13*, *B2*) and selected somatic hybrids and their progeny (*lanes A1-11*, *B3-13*). Each lane contains 10–15 μg of total DNA. The fragments in kilobases indicated on the *left* are tomato-specific fragments, and those on the *right* are those of *S. lycopersiocides*. The non-parental fragment is shown by an *arrow*

RFLP pattern. All of the 44 hybrids analyzed showed similar hybridization patterns. They contained 4 tomato-specific fragments of 14.0, 9.4, 4.0 and 3.2 kb, 2 *S. lycopersicoides*-specific fragments of 13.2 and 4.3 kb and 1 additional unique fragment(6.9 kb) not present in either parent. In contrast, the tomato 6.8 kb specific fragment and 2 *S. lycopersicoides*-specific fragments of 7.3 and 4.7 kb were absent in all the hybrids analyzed.

Discussion

Chloroplast DNA segregation in the hybrids

In earlier investigations the potential influence of different donor tissues to cpDNA segregation has been examined in somatic hybrids between tomato and *S. lycopersicoides*. Random or equal distribution of cpDNA was obtained when both fusion parents were derived from mesophyll cells (Li and Sink 1992). Moreover, as the heteroplasmic and unstable nature of the two types of chloroplasts resulted in rapid chloroplast segregation (Akada and Hirai 1986), the somatic hybrid plants were found to contain either one or the other parental chloroplast type (O'Connell and Hanson 1986; Donaldson et al. 1994; Wolters et al. 1995). The results of the present study agree well with those from previous reports. As a result of RFLP analyses, the cpDNAs segregated in an approximately 1:1 ratio, clearly indicating a random distribution pattern of chloroplasts despite the fact that the tomato cytoplasm was inactivated with 2.5 m*M* iodoacetamide (IOA) prior to the construction of somatic hybrids (Hossain et al. 1994). IOA has been reported to inactivate the cytoplasm of the nuclear donor protoplasts in some plants (Pelletier et al. 1988). However, in the present study it is not certain if it has affected chloroplast activity and the segregation of the chloroplast in the somatic hybrids.

One somatic hybrid had cpDNA restriction fragments corresponding to both parents. However, it was only detected in *Hae*III fragments hybridized with the Ba1-cloned fragment of tobacco and not in *Sac*I fragments. It seems, then, that recombination may occur only at the specific sites within the genome. The single probe covers only a limited area of the whole *Lycopersicon-Solanum* genome, hence, it is probable that these plants may contain small, undetectable proportions of cpDNA from the other parent, although it is generally accepted that there is always a predominance of one fusion parent in a hybrid. The presence of a recombinant chloroplast type has been observed in a few regenerants of intergeneric and interspecific hybrids between *L. esculentum* and *S. lycopersicoides* (Levi et al. 1988; Li and Sink 1992), *L. pennellii* (Wachocki et al. 1991) and *S. tuberosum* (Wolters et al. 1995).

Genome variation and constitution of the hybrids

After somatic hybridization, it is expected that the nuclear and cytoplasmic genomes of the fusion parents have combined to create a new variability in the hybrids (Austin et al. 1986; Derks et al. 1991; Xu et al. 1993). Changes in the genomic and genetic make-up may result not only in the apparent dominance of particular morphological traits of one parent but also create traits which are not expressed in both parents. In some cases, an expressed phenotype can be correlated with the ploidy level of the hybrids (Waara et al. 1991, 1992; Wolters et al. 1995). The vigorously growing tetraploid ($2n = 48$) and hexaploid ($2n = 72$) hybrid plants between tomato and *S. lycopersicoides* were easily discriminated from each other by some leaf traits inherent to each. The leaves of the hexaploid plants were broader and darker in color than those of the tetraploid plants. GISH analysis unequivocally revealed that the tetraploid plants had an equal number of chromosomes derived from each parent while the hexaploid plants contained an average of two sets of chromosomes from the tomato parent and one set from the wild *S. lycopersicoides* parent. The differences in chromosome complements or set of chromosomes inherited from each parent may have influenced the apparent variation in the leaf morphology. Probable correlation between the chromosomal composition of the aneuploid somatic hybrid plants produced by Hossain et al. (1994) and a specific phenotype has not been ascertained since they were no longer available during the course of this study.

Direct evidence of mitotic instability was observed in 2 of the somatic hybrid plants between tomato and *S. lycopersicoides* by the occurrence of some chromosomal changes. Chromosomal aberrations such as insertion, translocation and non-disjunctions are likely to occur either during tissue culture or as a result of other factors related to the interaction between parental genomes. The manifestation of abnormalities in chromosomes under in vitro conditions within a number of generations from the moment of fusion until plant regeneration is a general phenomenon (Fukui 1986; Evans 1986). The observed aberrations, however, were few and could likely be attributed to the use of leaf mesophyll protoplasts in the production of the hybrids as these are known to undergo fewer chromosomal changes than those from callus and cell suspension culture, which might be further augmented by the genetic relatedness of the parental species. Among the true *Solanum* species, *S. lycopersicoides* has the strongest affinity to tomato (Taylor 1986; Melchers 1992) despite the apparent difference in sizes of their chromosomes as confirmed in the present study. The available evidence, however, should be further studied in advanced progeny (either from sib-crosses or backcrosses) of the somatic hybrids. This would substantiate the results reported here and explain the phylogenetic relations between tomato and *S. lycopersicoides*.

Analyses of DNA variation using genome-specific repetitive sequences of nuclear DNA (Moore and Sink 1988; Zhao et al. 1989) and cDNA and single-copy DNA probes (Moore and Sink 1988; Wijbrandi et al. 1990; Derks et al. 1992; Xu and Pehu 1993) have proved to be useful in elucidating the genetic differentiation of many plants. In the present study, molecular characterization based on restriction fragment length polymorphism between the fusion parents showed that all of the somatic hybrids analyzed had similar RFLP patterns, which signifies that there is no conspicuous major variation among them in the loci under study. However, the loss of some parent-specific alleles and the presence of a few non-parental alleles were revealed, indicating rearrangement and/or recombination of the nuclear DNA. The number of recombinants could probably increase if more probes were used in the analysis.

In conclusion, it is evident that GISH and RFLP together are highly useful tools for the analysis of the genetical characteristics of the somatic hybrid plants between tomato and *S. lycopersicoides*. From the results of the present analysis , it appears that most of the hybrid plants developed by somatic hybridization have a considerably stable genetic composition. Although hybrids containing the complete chromosome set of both parents are not the main goal in plant breeding, the production of viable hybrids is considered here even more important to produce an advanced population, considering the formidable male sterility of the hybrids obtained from a sexual cross. Research studies on the potential utilization of the seed-producing somatic hybrids in tomato breeding are currently underway.

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