Theor Appl Genet (1990) 79: 193–200

THEORETICAL AND APPLIED GENETICS

© Springer-Verlag 1990

Molecular analysis of the extent of asymmetry in two asymmetric somatic hybrids of tomato

J. M. Melzer and M. A. O'Connell

Plant Genetic Engineering Laboratory and Agronomy and Horticulture Department, New Mexico State University, Las Cruces, NM 88003, USA

Received July 24, 1989; Accepted October 3, 1989
Communicated by P. Maliga

Summary. Two somatic hybrid plants generated from a single fusion event between *Lycopersicon esculentum* and irradiated *L. pennellii* protoplasts have been analyzed at the molecular level. Over 30 loci have been analyzed using isozymes and RFLPs. All loci tested on chromosomes 2–10 were heterozygous, while those loci on chromosome 12 were homozygous *L. pennellii* in both somatic hybrids. In one of the somatic hybrids, 2850, loci on chromosome 1 were also homozygous *L. pennellii*. The other somatic hybrid, 28F5, was heterozygous at all chromosome 1 loci tested, but exhibited altered stoichiometry of parental bands as compared to the sexual hybrid. Loci on chromosome 2 from both somatic hybrids have altered stoichiometry, with *L. pennellii* alleles being four times more abundant than expected. Both somatic hybrids contain the *L. esculentum* chloroplast genome, while only *L. pennellii* polymorphisms have been detected in the mitochondrial genome.

Key words: *Lycopersicon esculentum* – Asymmetric somatic hybrids – Protoplast fusion – Restriction fragment length polymorphism

Introduction

Fusing somatic cell protoplasts from different species produces novel genotypes which often cannot be produced via sexual hybridization. In fusions of protoplasts from phylogenetically distant species, chromosome elimination is often observed and, frequently, regeneration of plants from such fusions is dependent on partial elimination of chromosomes from one species (Harms 1983 a, b). Fragmentation of DNA by ionizing radiation may also result in chromosome elimination as well as in deletions and exchanges; radiation increases the frequency of ring chromosomes, bridges and acentric fragments (Evans 1974). Evidence of these events comes from cytological observation of metaphase and anaphase plates of irradiated cells. A practical application of directed chromosome elimination has been the use of radiation to generate asymmetric somatic hybrids (Gupta et al. 1984; Bates et al. 1987; Imamura et al. 1987; Sídorov et al. 1987; Yamashita et al. 1989) and cybrids (for review, Galun and Aviv 1983).

Cytological analysis of asymmetric somatic hybrids has determined the chromosome number and, in a few cases where fusion parents have distinct chromosome morphology, the specific chromosome contribution from each parent may be determined. In fusions of *Arabidopsis thaliana* with *Brassica campestris* (Gleba and Hoffmann 1978), *Atropa belladonna* with *Nicotiana chinensis* (Gleba et al. 1982) and *A. belladonna* with *N. plumbaginifolia* (Gleba et al. 1988), cytological observation detected possible exchanges between the two species' chromosomes. These authors were unable to confirm exchanges in any of these fusions or to identify the chromosomes from each species involved in the possible exchanges.

In the genera *Lycopersicon* and *Solanum*, chromosome morphologies are not distinct enough in somatic cells for cytological analysis of somatic hybrids. However, a nearly saturated linkage map of *L. esculentum* has been produced, which maps more than 300 RFLP markers on all 12 chromosomes (Bernatzky and Tanksley 1986; Tanksley et al. 1988). Utilizing this map, Young and Tanksley (1989) have developed the concept of graphical genotypes. They used 70 mapped RFLP markers to determine the genotypes of 200 BC_1 plants from a *L. esculentum* × *L. chmielewskii* cross and represented them in graphical form. They also looked at F_2 plants from a *L. esculentum* × *L. pennellii* cross. Moore and Sink

(1988) utilized four RFLP markers known to be located on chromosome 2 to analyze this chromosome in somatic hybrids of *L. esculentum* fused with *Solanum lycopersicoides*.

We have been attempting to create asymmetric somatic hybrids in tomato, using gamma-rays to induce chromosome elimination and fragmentation (O'Connell and Hanson 1985, 1987; O'Connell et al. 1986). In this paper we describe the use of mapped isozyme and RFLP markers for the molecular characterization of two asymmetric somatic hybrids generated from the fusion of *L. esculentum* cv UC82B with 3-krad-irradiated *L. pennellii* protoplasts. The regeneration and initial characterization of plants from this fusion were previously described (O'Connell and Hanson 1987).

Materials and methods

Plant material

Seeds of *L. pennellii*, LA 716, collected in Atico, Peru, were generously provided by C. Rick, Tomato Genetics Stock Center, University of California, Davis. Seeds of *L. esculentum* cultivar UC82B were from Petoseed Co. Somatic hybrids MOC 1732850F5 and MOC 17328F5 were isolated from a single regenerating callus generated from a fusion of 3-krad-irradiated *L. pennellii* and *L. esculentum* cultivar UC82B protoplasts, as described previously (O'Connell and Hanson 1987).

DNA isolation and DNA probes

Total DNA was isolated from lyophilized leaf tissue as described by Murray and Thompson (1980), except that DNA was diluted with four vol. of sterile distilled water and precipitated after cesium chloride centrifugation rather than undergoing dialysis. Chloroplast DNA (cpDNA) of *L. esculentum* was isolated following the protocol of Saltz and Beckman (1981). DNA to be used as probes was either nick-translated (Rigby et al. 1977) or oligolabelled (Feinberg and Vogelstein 1983) with α^{32}P-GTP. The nuclear DNA composition of somatic hybrids was analyzed using gel-purified fragments of mapped tomato cDNA clones (Bernatzky and Tanksley 1986) as probes. cpDNA was analyzed using oligolabelled *L. esculentum* cpDNA. Clone 2D4, a 2.1-kb *L. pennellii* mitochondrial SalI fragment which contains no known coding sequence but occurs in multiple copies in the mitochondrial genome (McClean and Hanson 1986), was used to analyze mitochondrial DNA (mtDNA).

Southern and hybridization conditions

Restriction enzyme digests were of 10 µg total DNA for at least 4 h at 37 °C. Electrophoresis, Southern transfer, hybridization and washes have been previously described (Melzer et al. 1989). Quantitation of autoradiograph images was performed with a laser densitometer.

Isozyme analysis

Approximately 0.5 g (wet weight) of leaf tissue was ground in 0.12 *M* reduced glutathione, adjusted to pH 7.6, with 1 *M Tris*. Samples were kept on ice during processing. Samples were absorbed into paper wicks and electrophoresed on starch gels and stained as described in Bernatzky and Tanksley (1986).

Chromosome counts and pollen staining

Root tips were collected from rooting shoots, tips were incubated for 4 h at 18 °C in 2 m*M* 8-hydroxyquinoline. Tissue was hydrolyzed 20 min in 4 *N* HCl at room temperature, rinsed with water, stained with acetocarmine and squashed. Viable pollen was determined after staining as described by Alexander (1969).

Results

Selection and morphology

Previously, we had observed that the differences in leaf shape of hybrids between *L. esculentum* and *L. pennellii* were indicative of increased ploidy (O'Connell et al. 1986). Most of the regenerants scored as somatic hybrids using isozyme markers had a leaf shape typical of a tetraploid (O'Connell and Hanson 1987). However, one regenerant had a leaf shape typical of a diploid. Somatic hybrids MOC 1732850F5 and MOC 17328F5 (hereafter referred to as 2850 and 28F5, respectively) were selected for detailed molecular analysis based on their morphology. Somatic hybrid 2850 was similar to the sexual F_1 (Fig. 1), having relatively thin leaves, intermediate leaf shape and open growth habit. Flowers had non-exserted stigmas like *L. esculentum*, but the corolla tube was somewhat enlarged. Overall, the morphology of 2850 suggested it was not tetraploid. Somatic hybrid 28F5, which was a regenerant from the same callus (i.e. same fusion event) as 2850, had characteristic tetraploid morphology with thick, round, wrinkled leaves, dense growth habit and exserted stigmas.

Cytological characterization and fertility

Inspection of root-tip squashes of either parent showed the expected diploid count of 24 chromosomes. Chromosome counts of 2850 revealed 44 ± 2 chromosomes; this was surprising since this individual had the leaf shape typical of a diploid. Counts of 28F5 were not possible

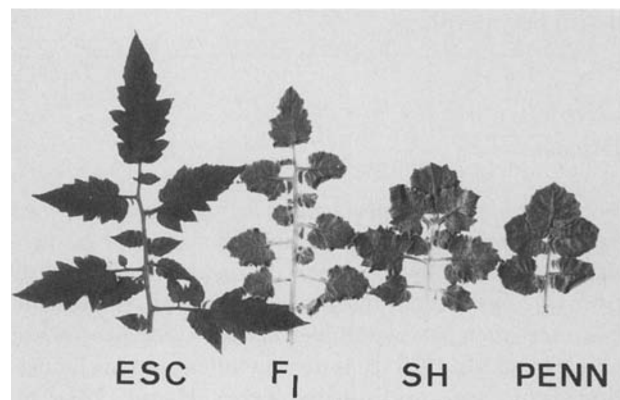

Fig. 1. Comparison of leaf shape of somatic hybrid 2850 with fusion parents. Genotypes are as indicated

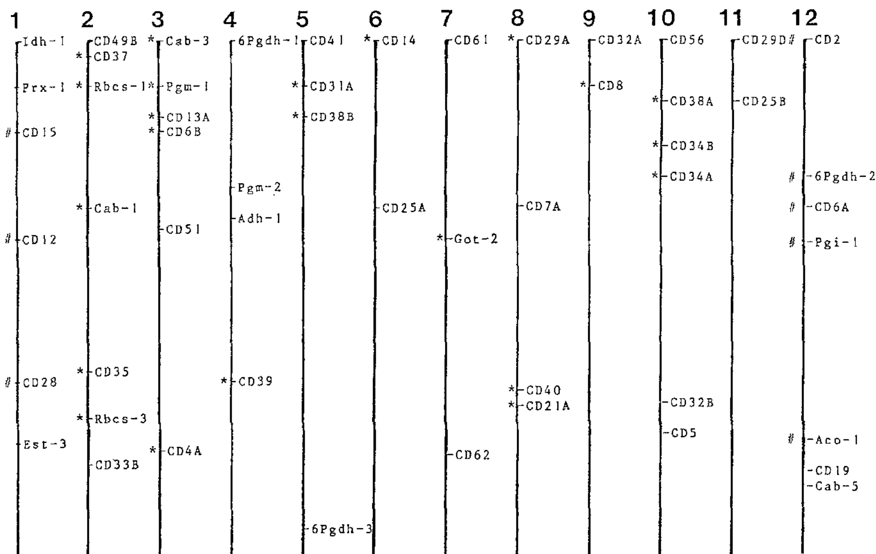

Fig. 2. Partial isozyme and RFLP map of somatic hybrid 2850. * indicate heterozygous loci in somatic hybrid 2850. # indicate homozygous *L. pennellii* loci in somatic hybrid 2850

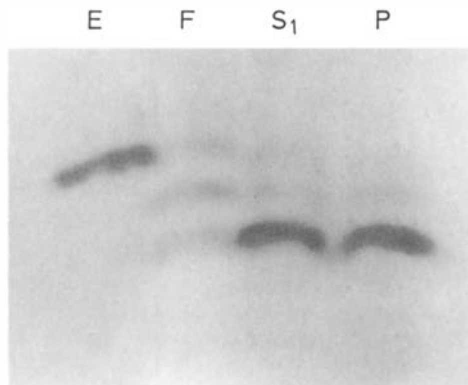

Fig. 3. Starch gel analysis of Pgi-1 activity. *Lanes E, P, F* and *S1* contain *L. esculentum, L. pennellii,* the sexual hybrid and somatic hybrid 2850, respectively

due to root-rot infection which eventually killed this plant. Pollen viability for 2850 was 4.4%. Somatic hybrid 2850 readily set fruit but not seed. Extensive crossing with *L. esculentum* resulted in a single viable seed. Germinated in vitro, the seedling was grossly abnormal, the shoot exhibited no apical dominance and produced a typical witches-broom morphology.

Isozymes and RFLP analysis

Despite having a morphology associated with a diploid hybrid, somatic hybrid 2850 had close to a tetraploid number of chromosomes, 44. In order to determine if there was a specific chromosome deletion responsible for this unusual phenotype, the genotype of the two somatic hybrids, 2850 and 28F5, was determined using molecular markers. Thirty-one loci on 11 of the 12 tomato chromosomes were analyzed for 2850 (Fig. 2) while 13 loci on 11 chromosomes were analyzed for 28F5.

A cursory evaluation of the molecular markers indicated that both somatic hybrids were heterozygous at loci on chromosomes 2–10. We were unable to test chromosome 11, as we only had one cDNA clone mapped to this chromosome, and we were unable to score its polymorphism. A more detailed analysis of the loci on chromosomes 1 and 12 was performed, since the *L. esculentum* copies of these chromosomes were suspected to be missing on the preliminary screen.

Analysis of chromosome 12

Loci at both ends and the middle of chromosome 12 were tested in the somatic hybrids. Three isozymes which are mapped on chromosome 12, Pgi, Aco and 6-Pgdh, were homozygous for the *L. pennellii* allele in both somatic hybrids (Fig. 3). Two RFLP loci which map to chromosome 12, CD2 (Fig. 4) and CD6A, were homozygous for the *L. pennellii* banding pattern in both somatic hybrids. These analyses indicate that both of the *L. esculentum* homologues of chromosome 12 are missing in the two somatic hybrids.

Analysis of chromosome 1

Three RFLP loci, CD15, CD12 and CD28, were used to characterize the complement of chromosome 1 homologues in the two somatic hybrids. Somatic hybrid 2850 displayed only the *L. pennellii* hybridization pattern, indicating that both of the *L. esculentum* homologues for chromosome 1 were missing. Somatic hybrid 28F5 displayed both the *L. pennellii* hybridization pattern and a weak signal with the *L. esculentum* pattern for CD15 (Fig. 5). These signals were quantitated, and the densitometric analysis indicated that the *L. esculentum* band was five times less abundant in 28F5 than the corresponding band in the F_1 hybrid.

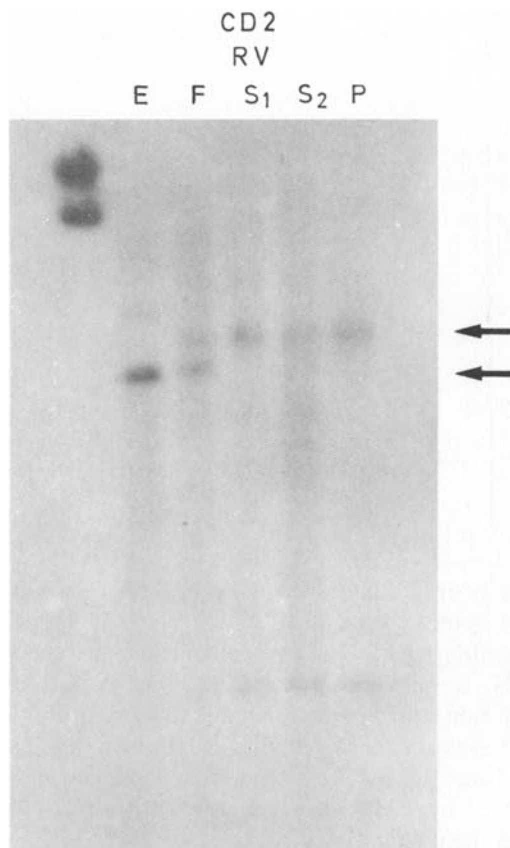

Fig. 4. Autoradiogram of total DNA probed with cDNA clone 3-17 encoding the chromosome 12 gene CD2. All *lanes* contain 10 μg Eco-RV-digested DNA. *Lanes E, P, F, S1* and *S2* contain *L. esculentum, L. pennellii,* the sexual hybrid, and somatic hybrids 2850 and 28F5, respectively. Bands of interest are indicated by *arrow heads*

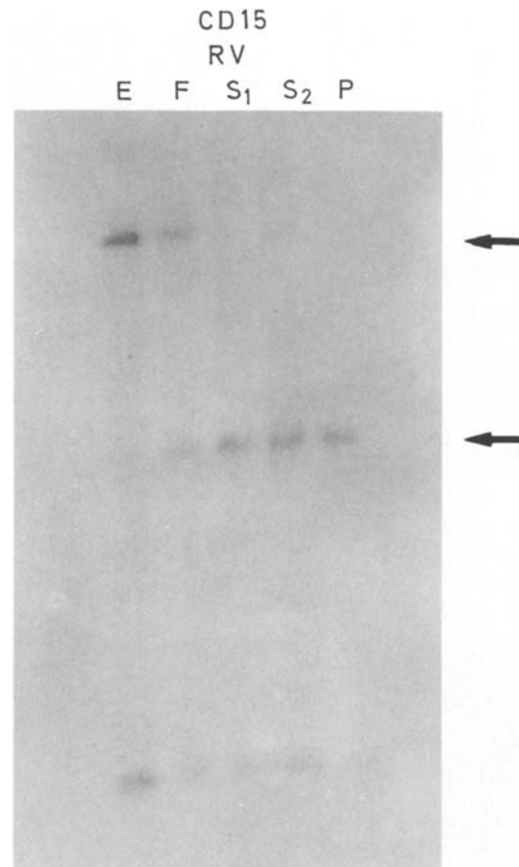

Fig. 5. Autoradiogram of total DNA probed with cDNA clone 3-82 encoding the chromosome 1 gene CD15. All *lanes* contain 10 μg EcoRV-digested DNA. *Lanes* are described in Fig.4. Bands of interest are indicated by *arrow heads*

Analysis of chromosome 2

Visual inspection of the isozyme gels and of the autoradiograms indicated that for most of the markers scored as heterozygous, the two parental alleles were present in equivalent amounts. This was not true for the loci mapped to chromosome 2. The loci, CD35, CD37, Cab-1, Rbcs-1 and Rbcs-3, all showed a stronger *L. pennellii* signal. The abundance of each parental banding pattern was quantitated, and the densitometric tracing indicated that the *L. pennellii* signal was four times more abundant than the corresponding band in the F_1 hybrid. Data for CD37 are shown in Fig. 6.

Organelle genomes

Both somatic hybrids 2850 and 28F5 contain only *L. esculentum* cpDNA, as detected in Southerns probed with labelled total cpDNA (Fig. 7). Both 2850 and 28F5 contain the 8.1-kb fragment unique to *L. esculentum* cpDNA and are missing the 5.8- and 2.2-kb fragments unique to *L. pennellii* cpDNA.

A cloned fragment of *L. pennellii* mtDNA, 2D4, which is present in several copies in both parental mitochondrial genomes (McClean and Hanson 1986), was chosen to approximate the nature of the mitochondrial genomes in the somatic hybrids. NruI, SalI, HindIII and EcoRI digests of total DNA were probed with nick-translated 2D4 (Fig. 8). None of the digests revealed any *L. esculentum*-specific bands. SalI and EcoRI digests showed *L. pennellii*-specific bands, with the SalI digest exhibiting altered stoichiometry in the banding pattern. The NruI and HindIII digests contained unique banding patterns distinct from either parental pattern.

The mitochondrial genomes in 2850 and 28F5 are not identical, since the hybridization patterns in the SalI and HindIII digests are different between these two individuals.

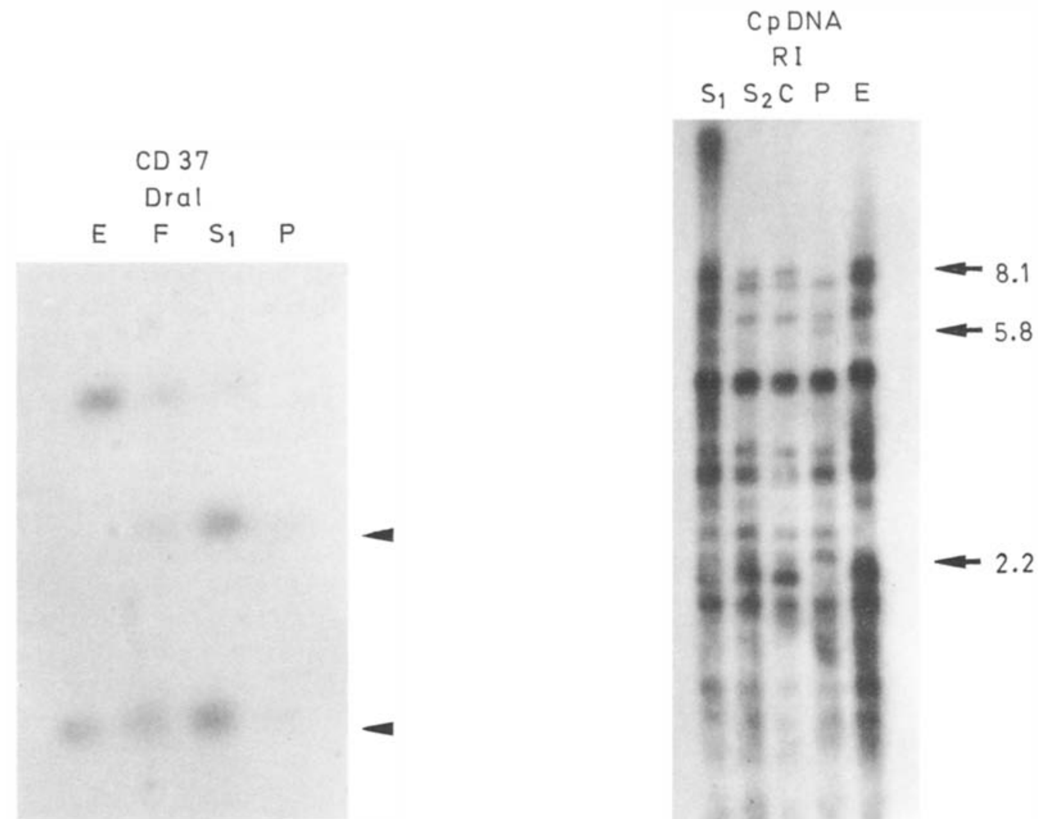

Fig. 6. Autoradiogram of total DNA probed with cDNA clone 3-288 encoding the chromosome 2 gene CD37. All *lanes* contain 10 μg DraI-digested DNA. *Lanes E, P, F* and *S1* contain *L. esculentum, L. pennellii,* the sexual hybrid and somatic hybrid 2850, respectively. Bands of interest are indicated by *arrow heads*

Fig. 7. Identification of chloroplast genomes present in somatic hybrids 2850 and 28F5. All *lanes* contain 4 μg EcoRI-digested total DNA. Nick-translated tomato chloroplast DNA was used as probe. *Lanes E, P, C, S1* and *S2* contain *L. esculentum, L. pennellii,* tomato cpDNA, and somatic hybrids 2850 and 28F5, respectively. *Arrow heads* indicate bands which differentiate *L. esculentum* and *L. pennellii*

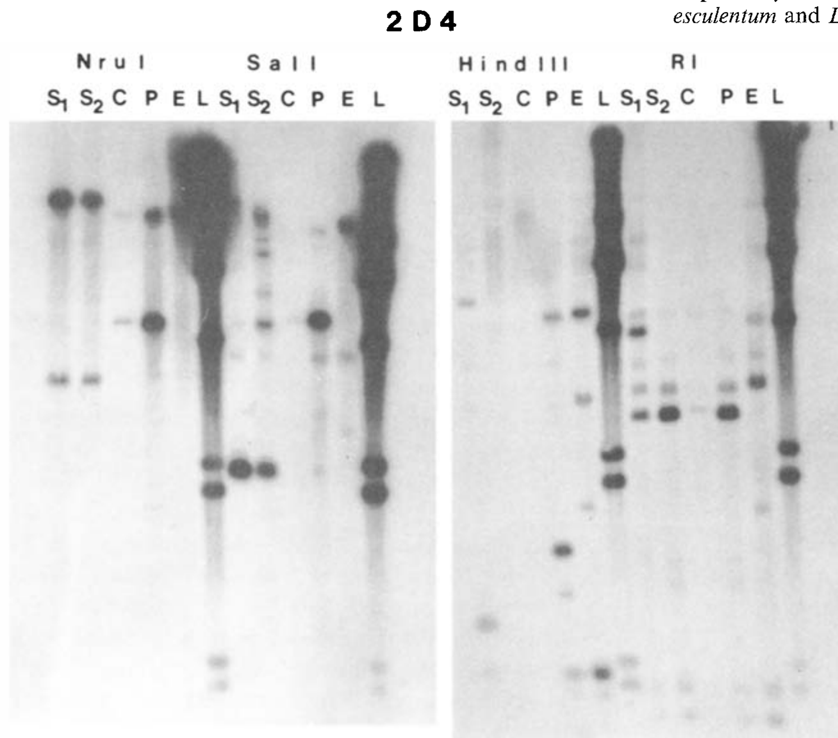

Fig. 8. Identification of mitochondrial genomes present in somatic hybrids 2850 and 28F5. All *lanes* contain 4 μg total DNA restricted with either NruI, SalI, HindIII or EcoRI. Hybridization was with mitochondrial probe 2D4. *Lanes* as in Fig. 7. *Lane L* contains HindIII-digested lambda DNA

Discussion

The fusion of *L. esculentum* and *L. pennellii* protoplasts and regeneration and initial characterization of 21 somatic hybrids were described previously (O'Connell and Hanson 1987). Using isozymes and RFLPs, we present a detailed molecular analysis of two of these regenerants, 2850 and 28F5, which regenerated from a single fusion product. Both somatic hybrids are asymmetric; they do not contain a full complement of chromosomes from each parent used in the fusion. Both 2850 and 28F5 have lost both copies of chromosome 12 of *L. esculentum*. In addition, 2850 has lost both copies of *L. esculentum* chromosome 1. The weak intensity of the *L. esculentum* signal in Southerns probed to look at the CD15 locus may indicate that 28F5 has lost one copy of *L. esculentum* chromosome 1 or is chimeric with respect to loss of chromosome 1.

Chromosome elimination is well documented in somatic hybrids, particularly in intergeneric and interfamilial fusions (Harms 1983a, b). One possible explanation for chromosome elimination is cell-cycle asynchrony. In the somatic hybrid parental species, chromosomes may not mix at metaphase, but assemble into blocks or clusters resulting in uneven segregation at mitosis (Kao et al. 1974; Constabel et al. 1977; Gosch and Reinert 1978). Asynchrony results when nuclei from rapidly dividing cells (suspension culture cells) and nuclei from slowly cycling or arrested cells (leaf mesophyll cells) are fused. Fusions of this type often result in intermediate cycling times (Weber et al. 1976; Gleba and Hoffmann 1978) and partial elimination or gross chromosomal alterations in chromosomes of the slower cycling parent (Kao et al. 1974; Constabel et al. 1977; Kao 1977; Chien et al. 1982; Pental et al. 1986). Elimination of chromosomes from callus cultures of somatic hybrids has been observed over several months (Kao 1977; Binding and Nehls 1978; Gleba and Hoffmann 1978; Krumbiegel and Schieder 1981; Chien et al. 1982). The elimination of *L. esculentum* chromosomes from somatic hybrids 2850 and 28F5 may reflect such an asynchrony of parental nuclei.

The weak signal of *L. esculentum* locus CD15 in Southerns of 28F5 might suggest 28F5 is chimeric with respect to loss of *L. esculentum* chromosome 1. Morphologically, 28F5 was uniform and did not exhibit variation in leaf morphology on different shoots. Complete loss of *L. esculentum* chromosome 1 is associated with certain morphological features, therefore, it is unlikely that 28F5 is chimeric as a result of regeneration from more than one cell with different *L. esculentum* chromosome 1 compositions. It is possible that *L. esculentum* chromosome 1 was undergoing elimination in planta.

Asynchrony of nuclei during mitosis is useful in explaining the loss of both copies of *L. esculentum* chromosomes from these somatic hybrids. Complete failure of chromosome 1 or 12 to line up on the metaphase plate would result in at least one daughter cell with no copies of that *L. esculentum* chromosome.

L. pennellii protoplasts used in these fusions were exposed to 3 krad of gamma-radiation. This is not a lethal dose in *L. pennellii* and we did not expect *L. pennellii* chromosome loss to occur. It is unlikely that the radiation treatment triggered some specific function in *L. pennellii* for elimination of foreign chromosomes.

The apparent amplification of *L. pennellii* chromosome 2 is of interest because the ribosomal RNA genes are located on this chromosome. Variation in ribosomal RNA gene copy number has been observed in tissue culture-derived plants of flax (Walbot and Cullis 1985), triticale (Brettell et al. 1986), potato (Landsmann and Uhrig 1985), *Nicotiana sylvestris* (De Paepe et al. 1983) and intergeneric tomato somatic hybrids (Moore and Sink 1988). In the intergeneric tomato somatic hybrids, three additional chromosome 2 loci indicated extra copies of this chromosome from the suspension cell protoplast parent *S. lycopersicoides* (Moore and Sink 1988). It is thought that the "stress" of tissue culture may trigger gene and possibly chromosome amplification. Why ribosomal RNA genes are more sensitive to this stress is unclear.

The karyotypes of the somatic hybrids correlate well with their morphology. Somatic hybrids 2850 and 28F5 differ in their copies of *L. esculentum* chromosome 1. Chromosome 1 contains at least one locus involved in the quantitative trait, leaf ratio (Tanksley et al. 1982). Therefore, the increased leaf thickness and rounder leaf outline in 28F5 compared to 2850 are likely a dosage effect of this chromosome 1 gene. Similarly, one of five QTLs for stigma exsertion was found on chromosome 1 (Tanksley et al. 1982). This QTL caused an effect opposite to the predicted parental effect, i.e. *L. pennellii* alleles at this locus resulted in reduced stigma exsertion. Somatic hybrid 2850 contains only *L. pennellii* chromosome 1 and has a non-exserted stigma.

Both somatic hybrids inherited *L. esculentum* cpDNA and predominantly *L. pennellii* mitochondrial DNA in the region surrounding 2D4 sequences. No evidence of cpDNA recombination was detected and sorting-out of chloroplasts appeared complete.

Inter- and intra-molecular recombination occurs in plant mtDNA (Lonsdale et al 1984; Shirzadegun et al. 1989), and recombination between parental mitochondrial genomes (i.e. inter-genomic recombination) has been demonstrated in somatic hybrids (Rothenberg et al. 1985; Rothenberg and Hanson 1988). Inheritance of one parental mitochondrial genome with no evidence of recombination has been observed as well (Shepard et al. 1983). Novel bands in the NruI and HindIII digests and altered stoichiometry of bands in the SalI digest suggest that the mtDNA may have undergone unusual recombi-

nation reactions. We cannot distinguish between inter- or intra-genomic recombination, as we have only analyzed a small percentage of the 300 to 400-kb mitochondrial genome.

Acknowledgements. This work was supported by USDA Grant No. 87-CRCR-1-2291.

References

Alexander MP (1969) Differential staining of aborted and non-aborted pollen. Stain Technol 44:117

Bates GW, Hasenkampf CA, Contolini CL, Piastuch WC (1987) Asymmetric hybridization in *Nicotiana* by fusion of irradiated protoplasts. Theor Appl Genet 74:718–726

Bernatzky R, Tanksley SD (1986) Towards a saturated linkage map in tomato based on isozymes and random cDNA sequences. Genetics 112:887–898

Binding H, Nehls R (1978) Somatic cell hybridization of *Vicia faba + Petunia hybrida.* Mol Gen Genet 164:137–143

Brettell RIS, Pallotta MA, Gustafson JP, Appels R (1986) Variation at the *Nor* loci in Triticale derived from tissue culture. Theor Appl Genet 71:637–643

Chien Y-C, Kao KN, Wetter LR (1982) Chromosomal and isozyme studies of *Nicotiana tabacum – Glycine max* hybrid cell lines. Theor Appl Genet 62:301–304

Constabel F, Weber G, Kirkpatrick JW (1977) Sur la compatibilité des chromosomes dans les hybrides intergeneriques de cellules de *Glycine max × Vicia hajastana.* CR Acad Sci Paris Ser D 285:319–322

De Paepe R, Prat D, Huguet T (1983) Heritable nuclear DNA changes in doubled haploid plants obtained by pollen culture of *Nicotiana sylvestris.* Plant Sci Let 28:11–28

Evans HJ (1974) Effects of ionizing radiation on mammalian chromosomes. In: German J (ed) Chromosomes and cancer. Wiley, New York, pp 191–237

Feinberg AP, Vogelstein B (1983) A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 132:6–13

Galun E, Aviv D (1983) Cytoplasmic hybridization: genetic and breeding applications. In: Evans DA, Sharp WR, Ammirato PV, Yamada Y (eds) Handbook of plant cell culture, vol 1. Macmillan, New York, pp 358–392

Gleba YY, Hoffmann F (1978) Hybrid cell lines *Arabidopsis thaliana + Brassica campestris:* No evidence for specific chromosome elimination. Mol Gen Genet 165:257–264

Gleba YY, Momot VP, Cherep NN, Skarzynskaya MV (1982) Intertribal hybrid cell lines of *Atropa belladonna × Nicotiana chinensis* obtained by cloning individual protoplast fusion products. Theor Appl Genet 62:75–79

Gleba YY, Hinnisdaels S, Sidorov VA, Kaleda VA, Parokonny AS, Boryshuk NV, Cherep NN, Negruiu I, Jacobs M (1988) Intergeneric asymmetric hybrids between *Nicotiana plumbaginifolia* and *Atropa belladonna* obtained by "gamma-fusion". Theor Appl Genet 76:760–766

Gosch G, Reinert J (1978) Cytological identification of colony formation of intergeneric somatic hybrid cells. Protoplasma 96:23–38

Gupta PP, Schieder O, Gupta M (1984) Intergeneric nuclear gene transfer between somatically and sexually incompatible plants through asymmetric protoplast fusion. Mol Gen Genet 197:30–35

Harms CT (1983 a) Somatic incompatibility in the development of higher plant somatic hybrids. Q Rev Biol 58:325–353

Harms CT (1983 b) Somatic hybridization by plant protoplast fusion. In: Potrykus I, Harms CT, Hinnen A, Hutter R, King PJ, Shillito RD (eds) Proc 6th Int Protoplast Symp, Basel, pp 69–84

Imamura J, Saul MW, Potrykus I (1987) X-ray irradiation-promoted asymmetric somatic hybridisation and molecular analysis of the products. Theor Appl Genet 74:445–450

Kao KN (1977) Chromosomal behaviour in somatic hybrids of *soybean – Nicotiana glauca.* Mol Gen Genet 150:225–230

Kao KN, Constable F, Michayluk MR, Gamborg OL (1974) Plant protoplast fusion and growth of intergeneric hybrid cells. Planta 120:215–227

Krumbiegel G, Schieder O (1981) Comparison of somatic and sexual incompatibility between *Datura innoxia* and *Atropa belladonna.* Planta 153:466–470

Landsmann J, Uhrig H (1985) Somaclonal variation in *Solanum tuberosum* detected at the molecular level. Theor Appl Genet 71:500–505

Lonsdale DM, Hodge TP, Fauron CM (1984) The physical map and organisation of the mitochondrial genome from the fertile cytoplasm of maize. Nucleic Acids Res 12:9249–9261

McClean PE, Hanson MR (1986) Mitochondrial DNA sequence divergence among *Lycopersicon* and related *Solanum* species. Genetics 112:649–667

Melzer JM, Warner RL, Kleinhofs A (1989) Regulation of nitrate reductase mRNA accumulation by nitrate and light. Mol Gen Genet 217:341–346

Moore PP, Sink KC (1988) Molecular analysis of single copy and repetitive genes on chromosome 2 in intergeneric tomato somatic hybrid plants. Plant Mol Biol 11:139–145

Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res 8:4321–4325

O'Connell MA, Hanson MR (1985) Somatic hybridization between *Lycopersicon esculentum* and *Lycopersicon pennellii.* Theor Appl Genet 70:1–12

O'Connell MA, Hanson MR (1987) Regeneration of somatic hybrid plants formed between *Lycopersicon esculentum* and *L. pennellii.* Theor Appl Genet 75:83–89

O'Connell MA, Hosticka LP, Hanson MR (1986) Examination of genome stability in cultured *Lycopersicon.* Plant Cell Rep 5:276–279

Pental D, Hamill JD, Pirrie A, Cocking EC (1986) Somatic hybridization of *Nicotiana tabacum* and *Petunia hybrida.* Recovery of plants with *P. hybrida* nuclear genome and *N. tabacum* chloroplast genome. Mol Gen Genet 202:342–347

Rigby P, Dieckman M, Rhodes C, Berg P (1977) Labelling deoxyribonucleic acid to high specific activity in vitro by nick translation with DNA polymerase I. J Mol Biol 113:237–251

Rothenberg M, Hanson MR (1988) A functional mitochondrial ATP synthase proteolipid gene produced by recombination of parental genes in a *Petunia* somatic hybrid. Genetics 118:155–161

Rothenberg M, Boeshore ML, Hanson MR, Izhar S (1985) Intergenomic recombination of mitochondrial genomes in a somatic hybrid plant. Curr Genet 9:615–618

Saltz Y, Beckman J (1981) Chloroplast DNA preparation from *Petunia* and *Nicotiana.* Plant Mol Biol Newslett 2:73–80

Shepard JF, Bidney D, Barsby T, Kemble R (1983) Genetic transfer in plants through interspecific protoplast fusion. Science 218:683–688

Shirzadegan M, Christey M, Earle ED, Palmer JD (1989) Rearrangement, amplification, and assortment of mitochondrial DNA molecules in cultured cells of *Brassica compestris.* Theor Appl Genet 77:17–25

Sidorov VA, Zubko MK, Kuchko AA, Komarnitsky IK, Gleba YY (1987) Somatic hybridization in potato: use of gamma-irradiated protoplasts of *Solanum pinnatisectum* in genetic reconstruction. Theor Appl Genet 74:364–368

Tanksley SD, Medina-Filho H, Rick CM (1982) Use of naturally occurring enzyme variation to detect and map genes controlling quantitative traits in an interspecific backcross of tomato. Heredity 49:11–25

Tanksley SD, Miller JC, Paterson A, Bernatzky R (1988) Molecular mapping of plant chromosomes. In: Gustafson JP, Appels R (eds) Chromosome structure and function. Plenum Press, New York, pp 157–173

Walbot V, Cullis CA (1985) Rapid genomic change in higher plants. Annu Rev Plant Physiol 36:367–396

Weber G, Constable F, Williamson F, Fowke L, Gamborg OL (1976) Effect of preincubation of protoplasts on PEG-induced fusion of plant cells. Z Pflanzenphysiol 79:459–464

Yamashita Y, Terada R, Nishibayashi S, Shimamoto K (1989) Asymmetric somatic hybrids of *Brassica*: partial transfer of *B. campestris* genome into *B. oleracea* by cell fusion. Theor Appl Genet 77:189–194

Young ND, Tanksley SD (1989) Restriction fragment length polymorphism maps and the concept of graphical genotypes. Theor Appl Genet 77:95–101