Mol Gen Genet (1982) 186:453–459

MGG

Restriction Endonuclease Analysis of Plastid DNA from Tomato, Potato and Some of Their Somatic Hybrids

Barbara Schiller[1], R.G. Herrmann[1], and G. Melchers[2]

[1] Botanisches Institut der Universität, D-4000 Düsseldorf 1, Federal Republic of Germany
[2] Max-Planck-Institut für Biologie, D-7400 Tübingen, Federal Republic of Germany

Summary. The buoyant density and endonuclease restriction patterns of potato (*Solanum tuberosum* L.) and tomato (*Lycopersicon esculentum*) ptDNA were examined and compared with those of their somatic hybrids. The plastids from these plants, both of which belong to the family of Solanaceae, contain a single DNA species whose density of 1.697 gcm^{-3} and size of approximately 156 kbp are similar to those of ptDNA from other higher plants. The Sal I restriction patterns were indistinguishable; however, those obtained with Kpn I, Pst I, and Eco RI disclosed that each species possesses a unique ptDNA. These observations suggest a relatively recent divergence of both species. Of the twelve hybrid lines screened, eight contained exclusively potato ptDNA and four contained only tomato ptDNA at a 0.1–3% level of detection. Rearrangements or modifications of DNA were not detected. The plastid identities of three hybrid lines that had previously been analyzed by isoelectric focusing of RuBPcase subunits (Melchers et al. 1978) agreed with those determined by restriction endonuclease analysis.

Introduction

Somatic hybrids of higher plants have been obtained by protoplast fusion between organisms that vary in their degree of relatedness. When the parental material is combined sexually or asexually to yield fertile hybrids, the hybrid character can generally be identified by karyotypic, morphological or – in case of haploids – by genetic analysis (Melchers and Labib 1974). However, biochemical analysis is generally required to certify the hybrid character of the fusion products that cannot be propagated sexually. Several somatic hybrids have been identified in this way and some of them even produced non-segregating progeny (reviewed in Schieder and Vasil 1980). Tomato-potato hybrids have not yet been produced by sexual crossing. To date, no fertile somatic hybrids have been identified either, perhaps because correct amphidiploids have not been found although some hybrid lines e.g., 2a/2a/36d approach this character (see Fig. 1). These somatic hybrids were originally obtained by fusion of mesophyll protoplasts of *Lycopersicon esculentum* Mill. var. *cerasiforme* (Dunal) Alef, mutant yellow green yg 6 (Rick) and protoplasts from a submersion callus culture of the originally dihaploid *Solanum tuberosum,* line HH 258 (Melchers 1978; Melchers et al. 1978). The hybrids could clearly be discerned from products of the tomato line because calli of this source material differentiated only roots under the applied conditions. However, since plants regenerated from the potato parent differed in their chromosome number and included tetraploids as well as several aneuploids (Wenzel et al. 1979) regenerated heteroploid potato could have been taken for hybrid phenotypes.

Isoelectric focusing patterns of RuBPcase, an abundant enzyme of the chloroplast stroma whose large and small subunits can serve as markers for plastome and genome, respectively (reviewed in Bottomley 1980), had unequivocally established that at least nine morphologically fairly distinct hybrid products, classified on phenotypic and karyotypic grounds, were created (Melchers et al. 1978; Poulsen et al. 1980, see also Table 2). Tomato and potato possess isoelectric variants for the small, nuclear-coded subunit and the hybrid regenerates exhibited the components from both parents demonstrating that they represented genuine nuclear hybrids. But only the large subunit of one parental line, either of tomato or potato, could be detected (Melchers et al. 1978; Poulsen et al. 1980) suggesting the existence of two hybrid categories, "pomatoes" (with plastids of potato) and "topatoes" (with plastids of tomato; Melchers 1980a, b).

With the electrofocusing technique, however, less than 10% plastid-coded protein of tomato or potato would not have been detected with certainty within the hybrids. Restriction endonuclease analysis which has been effective in identifying plastome differences even among closely related species (e.g., Atchison et al. 1976), offers a useful and more sensitive approach to investigate the reasons for the virtual absence of one of the parental large subunit types in somatic hybrids. In principle, this absence could be caused either by loss of one parental plastid type, by differential plastome expression or by DNA rearrangements which resulted in elimination of one LSU gene type. We have, therefore, examined the products obtained from the ptDNAs of the parental tomato and potato lines and some of the somatic hybrids using a variety of restriction endonucleases to distinguish between these alternatives. Progress reports of this work have been presented (Melchers 1980a, b).

Offprint requests to: G. Melchers, Max-Planck-Institut für Biologie, POB 2109, 7400 Tübingen, FRG

Abbreviations used in the text: ptDNA = plastid DNA, chloroplast DNA; cDNA = copy DNA; RuBPcase = ribulose bisphosphate carboxylase/oxygenase; LSU = large subunit of RuBPcase; kbp = kilobase pairs; SDS = sodium dodecyl sulfate; SSC = standard saline citrate; IEF = isoelectric focusing

0026-8925/82/0186/0453/$01.40

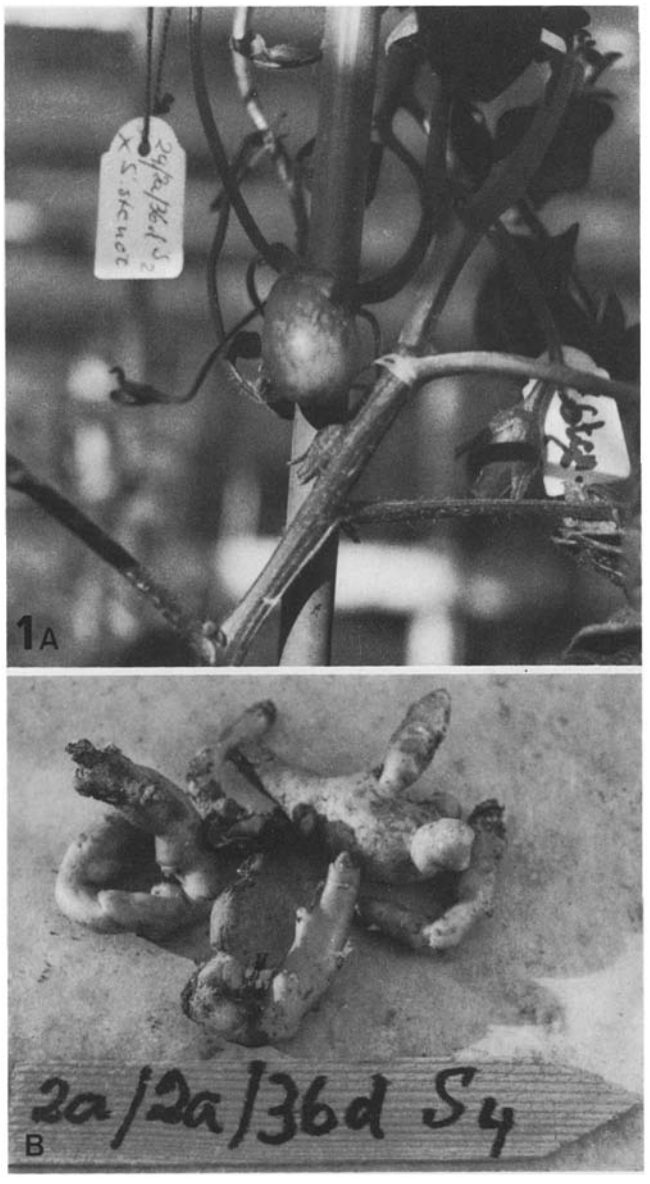

Fig. 1. Fruit (**A**) and "tubers" (**B**) of the somatic hybrid line 2a/2a/36d S2 and S4 "S_2, S_4, S_5 morphologically" very similar, S_1, S_3 similar but different from the others. The fruit was obtained after pollination with *Solanum stenotomum*. The wooden label measures 11 cm (76% natural size)

Material and Methods

Material

Twelve somatic intergeneric hybrid progenies of dihaploid potato (*Solanum tuberosum*, stock HH 258, 2n = 24; protoplasts from submersion callus culture with altered chromosome number due to changes during the callus growth period, Wenzel et al. 1979) and tomato (*Lycopersicon esculentum* var. *cerasiforme* mutant yellow-green yg 6, 2n = 24) have been studied along with their parental strains. Characteristics, nomenclature, fusion and selection methods, RuBPcase analysis as well as propagation of the material have been detailed previously (Melchers 1978; Melchers et al. 1978; Poulsen et al. 1980). All strains with identical numbers in the first part of their signature (e.g., 2a/1y/7c) derived from the same petridish with several calli (indicated here by "2a"). This does not necessarily imply that one callus originates from one fusion product. The notations "1y" and "7c" refer to further transfers of the callus. S_1, S_2 etc. denote different shoots of the same callus (examples see Figs. 1 and 4). The plants were cultivated on soil in a greenhouse with the exception of strain 7a/20e which was grafted onto tomato stocks (cultivar Supravite, Enza Zaden, Enkhuizen, Holland). The plant material used in this work was not selected for any particular features, e.g., hybrids with nearly amphidiploid chromosome numbers, tuber or fruit setting but included three lines previously used in the RuBPcase analysis (Melchers et al. 1978). Before leaf collection, the material was placed into darkness for 48 h to reduce its starch content.

Chloroplast Preparation, DNA Isolation. Unbroken chloroplasts were isolated by the rapid procedure of Walker (see Schmitt and Herrmann 1977) and further purified by centrifugation in continuous sucrose gradients (15–60%). The organelles were lysed in isopycnic sucrose to reduce shear forces, and high molecular DNA was obtained by a procedure involving Proteinase K (Merck), phenol – SDS – sodium dodecylsarcosinate extraction, isobutanol concentration (Stafford and Bieber 1977) and ethanol precipitation. The DNA was finally dissolved in a small volume of H_2O and stored at 4° C (for methods see Herrmann 1982).

DNA Restriction Agarose Gel Electrophoresis. The restriction endonuclease Eco RI was purchased from Boehringer (Mannheim). Sal I, Pst I and Kpn I were prepared according to Arrand et al. (1978). PtDNA (0.5–2.0 µg per assay) was digested and the resulting fragments were fractionated by agarose gel electrophoresis as detailed previously (Herrmann et al. 1980a). Lambda DNA, the Eco RI or Hind III digests of lambda DNA (all Boehringer) and Sal I digests of spinach ptDNA were used as size standards and included in the ptDNA sample (Seyer et al. 1981). After completion of electrophoresis the ethidium bromide-stained gels were photographed through Kodak 22A filter onto Polaroid 665 film using short wave-length UV transillumination. Each band is designated by the capital letter of the respective enzyme and consecutively numbered in decreasing size.

Analytical Ultracentrifugation. The buoyant densities in CsCl equilibrium gradients of native, denatured and reassociated DNA samples were determined by centrifugation at 44,000 rpm, at 25° C for 20 h in a Beckman Model E analytical ultracentrifuge (Herrmann et al. 1975). DNA of *Micrococcus luteus* (= *lysodeikticus*), $\rho = 1.731$ g cm^{-3}, served as density marker.

Fragment Isolation, Radioactive Labelling, Membrane Filter Hybridization. The Kpn I fragments of the chimeric plasmid pWHsp 403 which contains the entire gene of the large subunit of RuBPcase of spinach ptDNA (Herrmann et al. 1980b; Whitfeld and Bottomley 1980) were fractionated in low-melting-point agarose gels (Seaplaque agarose, Marine Coll. Inc., Maine USA) and extracted from the liquefied agarose by the method described in Seyer et al. (1981).

Copy DNA from a spinach Kpn I/Bam HI secondary fragment (0.8 kbp) carrying part of the large subunit gene was prepared using alpha ^{32}P-labelled dCTP from Amersham (Rigby et al. 1977). The specific activity of the product was usually in the range of 10^8 cpm/µg DNA. Kpn I restric-

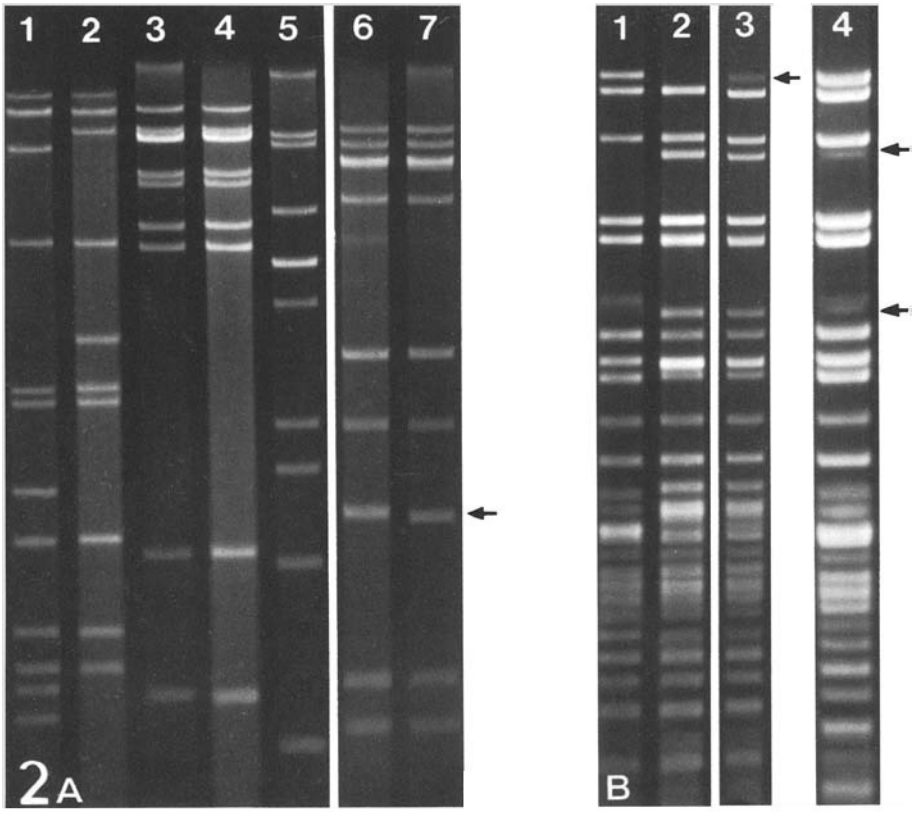

Fig. 2A, B. Fractionation of restriction endonuclease digests of ptDNA by slab gel electrophoresis; 0.5% (**A**) and 1% (**B**) agarose. **A** Fragment patterns obtained with Kpn I (tracks 1 and 2), Sal I (tracks 3–5) and Pst I (tracks 6 and 7). PtDNA of tomato (tracks 1, 3 and 6), potato (tracks 2, 4 and 7) and spinach (track 5). The diffuse band on top of tracks 3 and 7 represents contaminating nuclear DNA (cf. Herrmann 1982). Its occurrence varied from one preparation to another, and excess enzyme, prolonged incubation or addition of Triton X-100 did not affect the relative position or intensity of this zone in a given sample. **B** Eco RI fragment patterns of ptDNA from tomato (track 1) and potato (track 2). Tracks 3 and 4 display mixtures of 3% tomato (arrow) plus 97% potato ptDNA and 0.3% potato (arrows) plus 99.7% tomato ptDNA, respectively. Track 4 is overloaded and taken from a different gel

tion fragments of various ptDNAs, fractionated on agarose slab gels, were transferred to nitrocellulose sheets (Southern 1975, Wahl et al. 1979), the filters dried and incubated overnight at 62° C with cDNA (Denhardt 1966). To reduce background the filters were pretreated with Denhardt's buffer containing 10 µg/ml yeast RNA (Boehringer). Following hybridization unbound label was removed by exhaustive washings in 3 × SSC containing 0.2% sodium dodecylsarcosinate. Hybridization was detected by autoradiography using Kodak X-Omat AR X-ray film and Quanta III intensifying screens (Du Pont).

Results

The ptDNAs of tomato and potato were characterized by three independent criteria: CsCl density equilibrium centrifugation, restriction endonuclease analysis and hybridization of a nick-translated plastid-specific DNA sequence of spinach to fragment patterns of the restricted Solanaceae ptDNAs.

Buoyant density analysis combined with denaturation and reassociation distinguishes ptDNA from bulk nuclear and mitochondrial DNA. DNA from the Solanaceae chloroplast fractions each yielded a single narrow component of buoyant density 1.697 ± 0.001 g cm^{-3}. This peak density corresponds to an average base composition of 37 mole-% G+C (Schildkraut et al. 1962). Upon heat denaturation, the DNA increased in density by 0.016 g cm^{-3}. After reassociation for 60 min in 2 × SSC at a DNA concentration of 40 µg/ml the material banded unimodally at the expected position close to that of native DNA (1.699 g cm^{-3}), whereas whole-cell DNA showed only a slight decrease under these conditions (native: 1.695 g cm^{-3}, reassociated: 1.707 g cm^{-3}, experiments not shown). Thus buoyant density analysis indicated a single component of moderate sequence complexity with an average base composition characteristic of ptDNAs from all vascular plants so far studied (Kirk and Tilney-Bassett 1978).

This finding was confirmed by restriction endonuclease analysis. Agarose gel electrophoresis of fragments produced by digestion of the Solanaceae ptDNAs with Sal I, Pst I, Kpn I and Eco RI is shown in Fig. 2. Complete digests with these four enzymes produced characteristic fragment patterns similar to those of other higher plant ptDNAs (e.g., Herrmann et al. 1980b). The fragments were usually generated in molar amounts and totaled about 156.6 kbp (98.6×10^6 dalton) in the Sal I, Pst I and Kpn I digests. This sequence complexity is of the same order as the contour lengths of circular DNAs reported from other higher plant chloroplasts (reviewed in Herrmann and Possingham 1980). Sizes, number and multiplicity of fragments for these three series are listed in Table 1. Because of the large number of small fragments no attempt was made to sum the molecular weights of the Eco RI fragments.
Hybridization to Kpn I fragments of ptDNA from four different species with ^{32}P-cDNA made on a 0.8 kbp Kpn I/Bam HI secondary fragment from spinach ptDNA bearing the 3′-terminal part of the nucleotide sequences for the large subunit of RuBPcase (Seyer et al. 1981) is illustrated by Fig. 3. This plastome gene is phylogenetically conserved (Bottomley 1980). In all instances homology between heterologous fragments of similar size was observed; there was no radioactive signal with any other fragments even after prolonged exposure of the X-ray film confirming that this DNA was of plastid origin and indicating both that hybridization was specific as well as that most of this gene is located on Kpn fragments of comparable size in all these DNAs (see Discussion).

Table 1. Size (in kbp) and stoichiometry (in brackets) of ptDNA fragments produced by three restriction endonucleases

Kpn I		Sal I	Pst I	
T	P	T/P	T	P
	33.8	27.4		25.0
	26.6 (2×)	24.8		20.8
20.2	22.3	21.5 (2×)		18.9 (2×)
13.0	12.8	17.1		18.4
	8.4	15.9		15.0
	7.0	12.6		7.9 (2×)
	6.6	11.4		5.95
5.0		4.1	4.6 (2×)	4.55 (2×)
	4.25	2.7		2.8
	3.4			2.5
	3.0			
2.8				
2.5				
	0.82 (2×)			
	0.71			
	0.54			
Sum 157.6		159	153.2	

T = tomato P = potato

Analysis of restriction digests demonstrated also that, while the plastomes of potato and tomato are distinct, they appear to be closely related (Figs. 2 and 3). The patterns resembled each other since many of the well separated bands are common to both species. The extent of this similarity excludes mere coincidence. On the other hand, apart from Sal I the variability of restriction patterns between the two species was apparent even with enzymes which cut relatively infrequently.

Digestion with Sal I gave 9 discrete bands in 0.5% agarose slab gels with sizes ranging from 2.7–27 kbp. The patterns obtained from potato and tomato ptDNA with this enzyme were indistinguishable. Similarly the Pst I patterns resembled each other with the exception of one band which was slightly displaced (Fig. 2A, arrow). Greater variation among the restriction patterns of both ptDNAs was observed with Eco RI and Kpn I. Kpn I generated 10 and 12 fragments from tomato and potato ptDNA, respectively. Eight of these, including a doublet (band 2), are common to both species. K-3 and K-7 in tomato are smaller than those of potato (K-3 and K-5), and two new bands are evident (K-11 and K-12). The combined molecular weights of the four bands K-3, K-7, K-11 and K-12, about 30.6 kbp, are equivalent to that of K-3 and K-5 in potato suggesting that either ptDNA of tomato contains two additional Kpn I sites or that two have been abolished in potato ptDNA. Digestion with Eco RI produced more complex patterns (more than 50 fragments; Fig. 2B). Differences include, for example, band 1 of tomato which is missing in potato and bands 3 and 6 of potato which have no counterpart in tomato.

The observed differences were sufficiently distinct to be used as phenotypic markers to study ptDNAs of the hybrids. The ptDNAs of the somatic hybrids were thus digested with Kpn I and Eco RI and the patterns obtained were compared with the corresponding patterns from parental ptDNA. Twelve different hybrid strains have been examined. The results, summarized in Table 2, show that

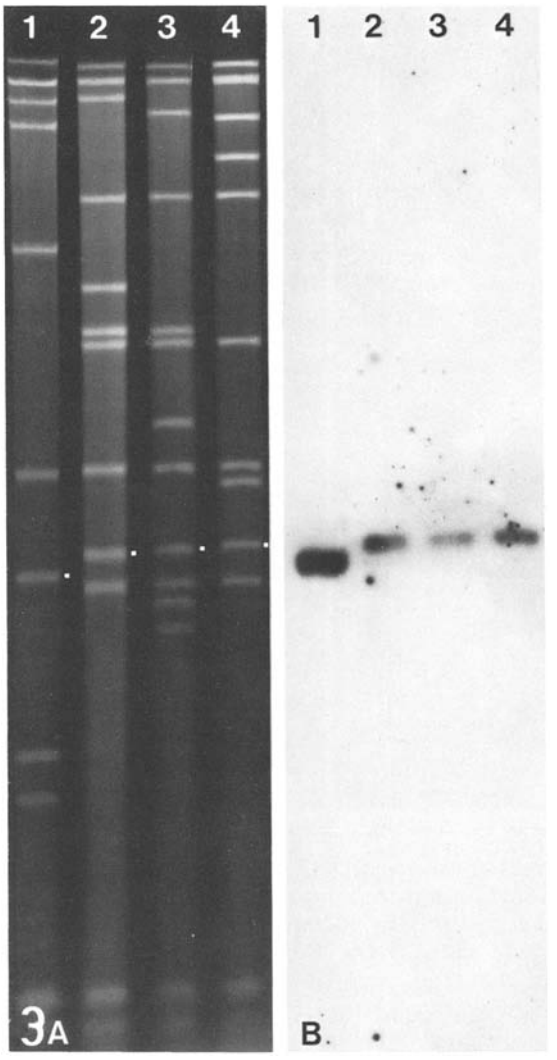

Fig. 3A, B. Detection of fragments of ptDNAs that contain sequences for the larger of the two subunit types of RuBPcase. **A** Cleavage patterns of different ptDNAs by endonuclease Kpn I: spinach (track 1), potato (track 2), tomato (track 3), tobacco (track 4). The entire fragment pattern was transferred from the 0.5% agarose slab gel to nitrocellulose sheets and hybridized to ^{32}P-labelled spinach gene sequence as described in Material and Methods. The corresponding autoradiograph is shown in part **B** (exposure 7 h). The hybridizing fragments are marked in part A

in each instance the restriction pattern of only one parent was obtained. None of the progenies possessed ptDNA from the second parent at the level of detection. This limit was determined by mixing appropriate ratios of the parental ptDNAs prior to endonuclease digestion (Fig. 2B). Provided sufficient DNA was available less than 0.1% admixture could be detected in overloaded gels. Also, no intermediate patterns indicative of rearrangements were observed suggesting that one of the parental plastid types was eliminated from the hybrid material during the development from fusion product to whole plant.

Discussion

The data presented above establish that the 1.697 g cm^{-3} DNA species from potato and tomato originates from plastids. Seventy or more percent of the isolated organelles ini-

Table 2. Karyological and biochemical analysis of somatic hybrids between tomato and potato

Strain		Chromosome number [a]	Small subunit of RuBPcase[a]	Large subunit of RuBPcase[a]	Plastid DNA (detection level of that of the second parent)
1b/2h/3	[c]	56	P+T	T	
1b/2j	S_{21}				T (P 1%)
1b/2j	S_{25}				T (P 0.5%)
2a/1y/7c	S_1	56			
2a/1y/7c	S_9	60			
2a/1y/7c	S_{13}[c] (Fig. 4)				P (T 0.5%)
2a/1y/7c	S_{14}	60	P+T	P	P (T 3%)
2a/1y/7c	S_{18}[c] (Fig. 4)				P (T 0.1%)
2a/1y/7c	S_{37}				P (T 3%)
2a/2a/36d	S_1	48 (49, 50)			T (P 1%)
2a/2a/36d	S_2 (Fig. 1)	49 (48)			
2a/2a/36d	S_3	49 (48)	P+T[b]	T	T (P 3%)
6a/1x/5d	S_6	56–57	P+T	P	
6a/4z/6g	S_2	57	P+T	T	
6b/1x/2a		50–52	P+T	T	
6b/2x/36	S_7				P
7a/1y/7c	S_{26}				P (T 3%)
7a/20e	S_2	72±2			P (T 1%)
7a/20e	S_6	72–74			
7a/20e	S_7[c]	72–74	P+T	P	P (T 3%)
7a/20e	S_{11}	~72			
7a/20e	S_{41}	72	P+T	P	
7c/13	S_{25}	58	P+T	P	
8a/22a	S_{51}	(58)≧90			

P = potato; T = tomato

[a] from Melchers et al. (1978)
[b] tryptic and chymotryptic peptide analysis (Poulsen et al. 1980)
[c] See Figs. in Melchers et al. (1978) and Poulsen et al. (1980)

tially possessed envelopes, the organelle fractions were essentially free of mitochondria and nuclei, and, after gradient centrifugation, nuclear fragments were only occasionally seen in the fluorescence microscope after staining with the sensitive fluorochrome DAPI (James and Jope 1978; Coleman 1979, cf. legend to Fig. 2). The assignment is reinforced by the similarity of physico-chemical features to ptDNA from other plants. For example, the size, equivalent to 156 kbp, is coincident with the contour lengths of circular ptDNA. The base composition of about 37 mole-% G+C (Kirk and Tilney-Bassett 1978), specific hybridization with a plastome-located gene as well as the relative cut frequencies of restriction endonucleases and characteristic size distributions of the resultant fragments are comparable (Fig. 3). We have recently demonstrated that Kpn fragment patterns of quite distant dicotyledons exhibit striking similarities (Herrmann et al. 1980b; Seyer et al. 1981, cf. Fig. 3). Therefore, it is probably no coincidence that common Kpn fragments between tobacco, tomato and potato (Fig. 3) lie in the conservative parts of the circular DNA molecule (Gordon et al. 1982; Dr. Salts, personal communication).

The tomato and potato ptDNAs can be distinguished by the patterns obtained with three restriction endonucleases. Since the summed recognition sites represent only a negligible portion of nucleotides of the circular DNA molecule (about 0.4% for the four enzymes used; cf. Table 1) data gathered from several enzymes were compared in order to obtain more reliable estimates of sequence alteration between the two DNAs and, if present, of those of their somatic hybrids. As with *Oenothera* and *Nicotiana* ptDNAs, Kpn I and Eco RI disclosed greatest variation among the chosen enzymes. However, most of the fragments are common to both species and the few variable ones appear to be related. The Sal I patterns were indistinguishable (Fig. 2A, tracks 3 and 4) while that of spinach ptDNA possesses almost no fragment coincident in size with those of potato and tomato (Fig. 2A, track 5). Taken together, this indicates a relatively recent evolutionary divergence of the tomato and potato plastomes. The shifted position of only one Pst band (Fig. 2A, tracks 6, 7 arrow) suggests the existence of deletions/insertions which appear to be a principal mechanism for plastome evolution (Herrmann et al. 1980b, Gordon et al. 1982).

Consistent restriction patterns of ptDNA were observed in the hybrids when compared with those of the parental species. Of twelve interspecific somatic hybrid progenies, three of which had been initially identified by nuclear hybridity (see Table 2), eight conformed exclusively to the potato pattern and four to the tomato parent at the 0.1–3.0% level of detection. RuBPcase analyses are available in three instances and these agree with data of the restriction patterns (Table 2; see Poulsen et al. 1980, Table 1). It should also be emphasized that recombinant or altered plastomes have been found neither by RuBPcase IEF patterns nor by restriction endonuclease analysis among those hybrid lines with identical nomenclature, even when their morphology differed considerably, e.g., 2a/1y/7c S13 versus S18 (Fig. 4). The latter example is of special interest, for line 2a/1y/7c S18 is characterized by chlorophyll deficiency and narrow leaves (Fig. 4; see Fig. 7 in Poulsen et al. 1980); nevertheless no difference in their plastomes could be detected. These findings are consistent with results of other laboratories (e.g., Chen et al. 1977; Belliard et al. 1978; Scowcroft and Larkin 1981) that demonstrated survival of only one or the other parental plastid type without apparent ptDNA recombination or rearrangement when fusion involves cells containing two diverse plastid populations.

A conserved distribution of restriction sites is apparent from comparative mapping on plastome DNAs of related *Euoenothera* or *Nicotiana* species; in tomato and potato

Fig. 4. Different characters of plants S13 and S18 originating from the same callus, hybrid line 2a/1y/7c. S13: normal green leaves. S18: chlorophyll deficient, narrow leaves. No difference in their plastomes was detectable (~1/2 natural size) see also Poulsen et al. (1978) Fig. 7.

it is evident from heterologous hybridizations (Fig. 3) as well as from double or triple restriction assays (experiments not shown). It precludes rearrangements over distances greater than 8 kbp, that is, beyond two adjacent cuts. Nevertheless rearrangement mechanisms that have been reported for mitochondrial DNA (e.g., Belliard et al. 1979) may also exist for plastids but not operate under somatic conditions. Changes in the order of homologous fragments (Palmer and Thompson 1981) and in the locations of thylakoid polypeptide genes (Bisanz, personal communication) have been found between ptDNAs of different dicotyledon species. However, it should be noted that attempts to demonstrate plastome recombination sexually in higher plants were unsuccessful even in *Oenothera* and *Pelargonium* where biparental transmission predominates (Stubbe, personal communication).

Taking into account the relatively low number of hybrids studied, the frequency distribution of plastomes of either parent among the hybrids is compatible with random selection. Table 2 compiles information available on this material. However, only when true amphidiploid hybrids exist, can the question be answered whether it is chance – via genetic drift – that determines whether one or the other plastome will prevail in hybrids with identical genomes. Somatic interspecific hybrids, especially between more distant organisms, sometimes suffer from rapid loss of chromosomes and vary in their extent of aneuploidy. If complementary genetic information for organelle biogenesis is lost by elimination of individual chromosomes or the intracellular genetic compartments disharmonize with increasing evolutionary disjunction of species or compete (Stubbe 1959; Sears 1980) two plastomes would not merely be sorted out by chance.

Acknowledgments. We thank Dr. Barbara Sears, Ms. Julia Morris, Dr. W. Bottomley and Dr. Helga Ninnemann for their help in the preparation of this manuscript, Ms. Monika Streubel and Ms. Inge Dobrigkeit for expert technical assistance. This research was supported by the "Deutsche Forschungsgemeinschaft".

References

Arrand JR, Myers PA, Roberts RJ (1978) A new restriction endonuclease from *Streptomyces albus* G. J Mol Biol 118:127–135

Atchison BA, Whitfeld PR, Bottomley W (1976) Comparison of chloroplast DNAs by specific fragmentation with EcoRI endonuclease. Mol Gen Genet 148:263–269

Belliard G, Pelletier G, Vedel F, Quetier F (1978) Morphological characteristics and chloroplast DNA distribution in different cytoplasmic parasexual hybrids of *Nicotiana tabacum*. Mol Gen Genet 165:231–237

Belliard G, Vedel F, Pelletier G (1979) Mitochondrial recombination in cytoplasmic hybrids of *Nicotiana tabacum* by protoplast fusion. Nature 281:401–403

Bottomley W (1980) Fraction I Protein. In: Reinert J (ed) Results and problems in cell differentiation. Chloroplasts, Vol 10. Springer, Berlin Heidelberg New York, pp 179–199

Chen K, Wildman SG, Smith H (1977) Chloroplast DNA distribution in parasexual hybrids as shown by polypeptide composition of fraction I protein. Proc Natl Acad Sci USA 74:5109–5112

Coleman AW (1979) Use of the fluorochrome 4'6-diamino-2-phenylindole in genetic and developmental studies in chloroplast DNA. J Cell Biol 89:299–305

Denhardt DT (1966) A membrane-filter technique for the detection of complementary DNA. Biochem Biophys Res Commun 23:641–652

Gordon KHJ, Crouse EJ, Bohnert HJ, Herrmann RG (1982) Physical mapping of differences in chloroplast DNA of the five wild-type plastomes in *Oenothera* subsection *Euoenothera*. Theor Appl Genet 61:373–384

Herrmann RG (1982) The preparation of circular DNA from plastids. In: Edelman M, Hallick RB, Chua N-H (eds) Methods in chloroplast molecular biology. Elsevier, North Holland, p 259–280

Herrmann RG, Possingham JV (1980) Plastid DNA – The Plastome. In: Reinert J (ed) Results and problems in cell differentiation, vol 10. Springer, Berlin Heidelberg New York, p 45–96

Herrmann RG, Bohnert HJ, Kowallik KV, Schmitt JM (1975) Size, conformation and purity of chloroplast DNA of some higher plants. Biochim Biophys Acta 372:305–317

Herrmann RG, Whitfeld PR, Bottomley W (1980a) Construction of a Sal I/Pst I restriction map of spinach chloroplast DNA using low-gelling temperature agarose electrophoresis. Gene 8:179–191

Herrmann RG, Seyer P, Schedel R, Gordon K, Bisanz C, Winter P, Hildebrandt JW, Wlaschek M, Alt J, Driesel AJ, Sears BB (1980b) The plastid chromosomes of several dicotyledons. In: Bücher Th, Sebald W, Weiss H (eds) Biological chemistry of organelle formation. 31st. Coll Mosbach. Springer, Berlin Heidelberg New York, p 97–112

James TW, Jope Ch (1978) Visualization by fluorescence of chloroplast DNA in higher plants by means of the DNA-specific probe 4'6-diamino-2-phenylindole. J Cell Biol 79:623–630

Kirk JTO, Tilney-Bassett RAE (1978) The Plastids. North Holland, Elsevier

Melchers G (1978) Potatoes for combined somatic and sexual breeding methods; plants from protoplasts and fusion of protoplasts of potato and tomato. In: Alfermann AW, Reinhard E (eds) Production of natural compounds by cell culture methods. GSF München, p 306–311

Melchers G (1980a) Protoplast fusion, mechanism and consequences for potato breeding and production of potato + tomatoes. In: Advances in protoplast research. Académiai Kiadó, Budapest, p 283–286

Melchers G (1980b) The somatic hybrids between tomatoes and potatoes. In: Sala F, Parisi B, Cella R, Cifferi O (eds) Plant cell cultures: Results and perspectives. Elsevier, North Holland, p 57–58

Melchers G, Labib G (1974) Somatic hybridization of plants by fusion of protoplasts. Mol Gen Genet 135:277–294

Melchers G, Sacristán MD, Holder AA (1978) Somatic hybrid plants of potato and tomato regenerated from fused protoplasts. Carlsberg Res Commun 43:203–218

Palmer JD, Thompson WF (1981) Rearrangements in the chloroplast genome of mung bean and pea. Proc Natl Acad Sci USA 78:5533–5537

Poulsen C, Porath D, Sacristán MD, Melchers G (1980) Peptide mapping of the ribulose bisphosphate carboxylase small subunit from the somatic hybrid of tomato and potato. Carlsberg Res Commun 45:249–267

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

Schieder O, Vasil IK (1980) Protoplast fusion and somatic hybridization. In: Vasil IK (ed) Perspectives in plant cell and tissue culture. Int Rev Cytol, Suppl 11b, Academic Press, New York London Toronto Sidney San Francisco, p 21–42

Schildkraut CL, Marmur J, Doty P (1962) Determination of the base composition of deoxyribonucleic acid from its buoyant density in CsCl. J Mol Biol 4:430–443

Schmitt JM, Herrmann RG (1977) Fractionation of cell organelles in silica sol gradients. In: Prescott D (ed) Methods in cell biology, vol 15. Academic Press, New York San Francisco London p 177–200

Scowcroft WR, Larkin PJ (1981) Chloroplast DNA assorts randomly in intraspecific somatic hybrids of *Nicotiana debneyi*. Theor Appl Genet 60:179–184

Sears BB (1980) Disappearance of the heteroplasmic state for chloroplast markers in zygospores of *Chlamydomonas reinhardtii*. Plasmid 3:18–34

Seyer P, Kowallik KV, Hermann RG (1981) A physical map of *Nicotiana tabacum* plastid DNA including the location of structural genes for ribosomal RNAs and the large subunit of ribulose bisphosphate carboxylase/oxygenase. Curr Genet 3:189–204

Southern EM (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 98:503–517

Stafford D, Bieber D (1975) Concentration of DNA solutions by extraction with 2-butanol. Biochim Biophys Acta 378:18–21

Stubbe W (1959) Genetische Analyse des Zusammenwirkens von Genom und Plastom bei *Oenothera*. Z Indukt Abstamm Vererbungsl 90:288–298

Wahl GM, Stern M, Stark GR (1979) Efficient transfer of large DNA fragments from agarose gels to diazobenzyloxymethyl-paper and rapid hybridization by using dextran sulfate. Proc Natl Acad Sci USA 76:3683–3687

Wenzel G, Schieder O, Przewozny T, Sopory SK, Melchers G (1979) Comparison of single cell culture derived *Solanum tuberosum* L. plants and a model for their application in breeding programs. Theor Appl Genet 55:49–55

Whitfeld PR, Bottomley W (1980) Mapping of the gene for the large subunit of ribulose bisphosphate carboxylase on spinach chloroplast DNA. Biochem Internat 1:172–178

Communicated by J. Schell

Received May 4, 1982