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Cytoplasmic Hybridization in *Nicotiana*: Mitochondrial DNA Analysis in Progenies Resulting from Fusion Between Protoplasts Having Different Organelle Constitutions

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Summary. Our previous studies indicated that fusion products with one functional nucleus but organelles of the two fusion partners (i.e. heteroplastomic cybrids) could be obtained by fusing X-irradiated (cytoplasmic donor) with non-irradiated (recipient) *Nicotiana* protoplasts. The present report deals with the analysis of mitochondria in cybrid populations resulting from the fusion of donor *Nicotiana tabacum* protoplasts with recipient protoplasts having a *N. Sylvestris* nucleus but chloroplasts of an alien *Nicotiana* species, and exhibiting cytoplasmic male sterility. The two fusion parents showed significant differences in restriction patterns of their chloroplast and mitochondrial DNA. Four groups of cybrid plants were obtained by this fusion. All had *N. sylvestris* nuclei but contained either donor or recipient chloroplasts and had either sterile or fertile anthers. There was no correlation between anther fertility and chloroplast type. The mitochondrial DNA restriction patterns of sterile cybrids were similar to the respective patterns of the sterile fusion partner while the mitochondrial DNA restriction patterns of the fertile cybrids were similar to the respective patterns of the fertile fusion partner. The results indicate an independent assortment of chloroplasts and mitochondria from the heteroplastomic fusion products.

Introduction

Maternal-uniparental transmission of plasmon elements is the common result of sexual hybridization in angiosperms. Evidence for regular biparental transmission of cell organelles is limited to a few genera and, in angiosperms, is presently restricted to plastids (Correns 1937; Tilney-Bassett 1978; Galun 1981). The recent development of methodologies leading from protoplast fusion to functional somatic hybrids in flowering plants (see: Schieder and Vasil 1980) paved the way for organelle genetics in this important group of organisms (Galun and Aviv 1978; Izhar and Power 1979; Gleba 1979). In the somatic hybridization technique developed by us (Zelcer et al. 1978; Aviv and Galun 1980) X-irradiated (donor) protoplasts are fused with non-irradiated (recipient) protoplasts leading to fusion products containing organelles from both partners and a nucleus from the recipient alone. Thus, a situation is achieved which is different from that existing in the fertilized egg cell of most angiosperms.

Recently we (Aviv and Galun 1980) fused X-irradiated (donor) protoplasts from normal, male-fertile, tobacco (*Nicotiana tabacum*) plants with (recipient) protoplasts from Type A plants. The Type A plants, which were obtained in a previous fusion experiment (Zelcer et al. 1978; Galun and Aviv 1979), have *N. sylvestris* nuclei, chloroplasts of an alien (Australian type *Nicotiana sp*) cytoplasm (termed: Line-92) and are cytoplasmic male sterile. Four groups of somatic cybrid plants were recognized: (1) male-fertile with *N. tabacum* chloroplasts; (2) male-fertile with Line-92 chloroplasts; (3) male-sterile with *N. tabacum* chloroplasts and (4) male-sterile with Line-92 chloroplasts. Of these groups (2) and (3) had novel combinations of plastids versus fertility/sterility, which did not exist in either donor or recipient protoplasts; cybrids of group (1) had the same combination as in *N. tabacum* (donor) but *N. sylvestris* nuclei, while group (4) was apparently identical to Type A plants but had resulted from a fusion event (the plants were isolated from calli which also regenerated cybrids having *N. tabacum* chloroplasts).

In the present work using the four groups of cybrids mentioned above, as well as the plants which served as the original fusion partners, we trace the assortment of both mitochondria and plastids by gel electrophoresis of organelle DNA which had been digested with restriction endonuclease. Two questions were asked: (1) Is there a correlation between mitochondrial type and male-sterility and (2) is there independent plastid and mitochondrial assortment in cybrids resulting from heteroplastomic fusion products? We note that it has been suggested that cytoplasmic male sterility in *Zea* (Pring and Levings 1978) and *Nicotiana* (Belliard et al. 1979) is controlled by genetic information located in the mitochondria.

Material and Methods

Plant Material

The plants used for mitochondrial (mt) DNA restriction analyses as well as their abbreviated designations are listed in Table 1. Abbreviated designations for nuclear/chloroplast/anther type composition follow our previous publication (Aviv and Galun 1980). The cybrids are numbered according to the original Petri plate (capital letter), serial callus number within each plate (first number) and serial number of plant regenerated from a given callus (second number). Male fertile cybrids were sexually propagated by self-pollination while male-sterile cybrids were pollinated with *N. sylvestris*. We found that the chloroplast/anther type composition was retained in the sexual progeny of the individual cybrids. In some of the male fertile cybrids, stamen filaments varied in length resulting in variability in the rate of self-pollination.

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Table 1. Plants utilized for mtDNA restriction analyses

Plants	Abbreviated designation (nucleus/chloroplasts/anther type)
I. Reference plant types	
N. tabacum cv Xanthi	*tab/tab/fert*
N. sylvestris	*syl/tab*[a]*/fert*
Line-92 (CMS- *N. tabacum*)	*tab/L-92/ster*
Type A (f-48)	*syl/L-92/ster*
II. Cybrids	
B-13-4	*syl/tab/fert*[b]
A-10-5	*syl/tab/fert*
A-10-7	*syl/tab/fert*
C-12-2	*syl/L-92/fert*
A-45-3	*syl/L-92/fert*
D-34-2	*syl/tab/ster*
A-6-3	*syl/L-92/ster*

[a] *N. sylvestris* chloroplasts are indistinguishable in all features tested from *N. tabacum* chloroplasts (D. Aviv and R. Fluhr, unpublished results)

[b] All plants designated *fert* had functional pollen but in some of these cybrids the stamens filaments were shorter than normal

The source of reference plants was described previously (Aviv and Galun 1980). *N. tabacum* cv Xanthi and *N. sylvestris* were maintained by self-pollination and Line-92 and Type A were propagated by pollination with *N. tabacum* cv Xanthi and *N. sylvestris*, respectively.

Preparation of Homogenates

Leaf Homogenates. Leaves (300–500 g) were harvested from 6–8 week old plants. All subsequent operations were at 4° C. After washing, the leaves were de-ribbed, cut into small squares and homogenized ($^3/_4$ maximal speed, Waring blendor) in Buffer A (0.5 M sucrose, 5 mM EDTA, 50 mM Tris-HCl, pH 7.5, 0.1% bovine serum albumin, 1 mM β-mercaptoethanol) at a 1:4 (W/V) ratio. The homogenate was passed through 4 layers of cheese cloth and 2 layers of Miracloth.

Cultured Cell Homogenates. Cell suspensions from leaf-explant derived calli were maintained as shake cultures in Murashige and Skoog (1962) medium (MS) containing β-naphthalene acetic acid (0.6 µg/ml) and 6-benzyl-aminopurine (0.2 µg/ml) at $26 \pm 2°$ C. A few days before use the cells were diluted 1:10 with MS and 200 ml suspensions incubated on a rotary shaker (100 rpm) in 500 ml Erlenmeyer flasks until a 40 to 60% packed volume was reached. Usually the contents of 6 flasks were used for each mtDNA extraction. The cells were harvested by filtration over Miracloth and resuspended in cold Buffer A at a ratio of 1:1 (W/V). All subsequent operations were at 4° C unless otherwise specified. The resuspended cells were disrupted in a French Pressure cell at 3,000 pounds per square inch, the homogenate adjusted to 1:4 (W/V) with Buffer A and passed through 2 layers of Miracloth.

Preparation of Mitochondrial Fractions

Mitochondrial fractions were prepared according to Pring and Levings (1978) with several modifications. These modifications were required to cope with green leaf material rather than with etiolated shoots, and were adopted after testing the resultant DNA by analytical ultracentrifugation.

Isolation of Crude Mitochondrial Fractions. The filtered homogenate was centrifuged (Sorvall GSA rotor 3100 rpm, 10 min) and the supernatant was recentrifuged (Sorvall GSA rotor, 10,000 rpm, 15 min) to obtain a mitochondrial pellet. The pellet was resuspended in Buffer B (Buffer A but containing 0.4 M sucrose and without β-mercaptoethanol) to a total volume $^1/_5$ that of the original homogenate, centrifuged (Sorvall SS-34 rotor, 3,500 rpm, 10 min) and the supernatant recentrifuged in the same rotor (8,500 rpm, 15 min). The pellet was rinsed, resuspended in a small volume of Buffer C (0.3 M sucrose, 50 mM Tris-HCl, pH 7.5) and centrifuged (Sorvall SS-34 rotor 8,700 rpm, 15 min) to provide the crude mitochondrial pellet. All procedures were carried out at 4° C.

DNase Treatment. The crude mitochondrial pellet was suspended in 5 ml Buffer C containing 10 mM $MgCl_2$ and 250 µg DNase I per ml (Calbiochem), and incubated at 4° C for 1 h. DNA digestion was terminated by addition of EDTA to a final concentration of 10 mM and the suspension was centrifuged (SS-34 rotor, 8,700 rpm, 15 min). The pellet was resuspended in Buffer C containing 10 mM EDTA and centrifuged (same conditions as above) two more times. The high DNase concentration was found in preliminary tests to be essential for removal of non-mitochondrial DNA from the crude mitochondrial pellet derived from green leaf homogenates.

Sucrose Gradient Purification. A discontinuous density gradient was prepared in nitrocellulose tubes with the following sucrose molarities: *1.8* (9.3 ml), *1.45* (9.3 ml), *1.2* (9.3 ml), *0.9* (4.5 ml) and *0.6* (4.5 ml). DNase-purified mitochondria were loaded on the gradients and the tubes centrifuged in a Beckman SW-28 rotor (20,000 rpm, 45 min, 4° C). Cytochrome c assays (Smith 1955) and electron microscope examinations located the mitochondria at the 1.2/1.45 M sucrose interface. The mitochondrial layer was gradually diluted 4.5-fold with 50 mM Tris-HCl (pH 7.5) and pelleted by centrifugation in a Sorvall SS-34 rotor (10,000 rpm, 20 min).

Mitochondrial DNA Purification

The purified mitochondrial pellet was suspended in 2.8 ml of TE Buffer (20 mM EDTA, 50 mM Tris-HCl, pH 8.0). Then, 0.25 ml of 0.1 M NaCl, 0.25 ml of 0.5 M EDTA (pH 8.0) and 0.47 ml 10% sarkosyl were added. The mixture was incubated with proteinase K (50 µg/ml) for 30 min at 37° C and 2.2 g CsCl added. This solution was underlayered with 3 ml CsCl (4 g in 3 ml TE Buffer) and centrifuged in a Beckman SW-40 rotor (28,000 rpm, 40 h, 18° C). The DNA-containing fractions were pooled, diluted in 4 volumes of 1 mM EDTA, 10 mM Tris-HCl (pH 8.0) and sedimented in the same rotor (38,000 rpm, 18 h, 18° C). The purified mtDNA pellet was resuspended overnight in 50–200 µl of the same dilution buffer.

Restriction Endonuclease Digestion and Gel Fractionation

Xho I and *Sal* I restriction endonucleases (New England Biolabs) were used as recommended by the manufacturer. Incubations were carried out for 6 h to ensure complete digestion. Overnight incubations did not affect the restriction patterns. Fractionation

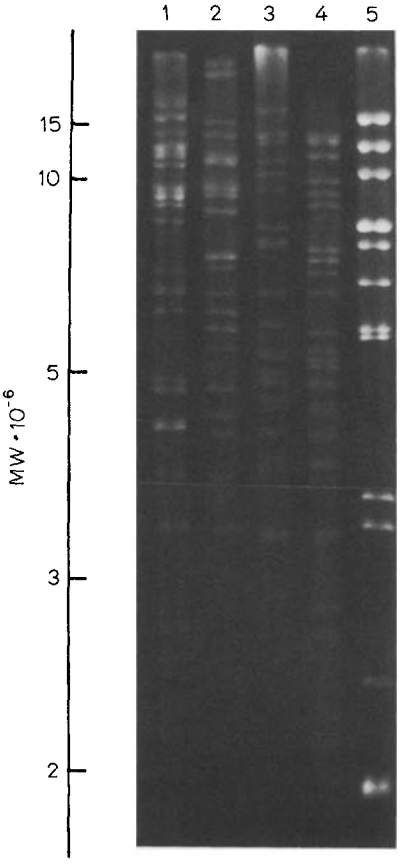

Fig. 1. Restriction endonuclease patterns of mtDNA from *Nicotiana tabacum* (lanes 1 and 3) and Line-92 (lanes 2 and 4) after digestion with *Sal*I (lanes 1 and 2) and *Xho*I (lanes 3 and 4). Mitochondria were isolated from green leaves. The *Xho*I restriction pattern of chloroplast DNA from *N. tabacum* (lane 5) is included to indicate the lack of detectable contamination of the mtDNA preparation by this source. Fragment molecular weights were estimated from the mobility of *Hin*dIII digestion products of λ phage DNA

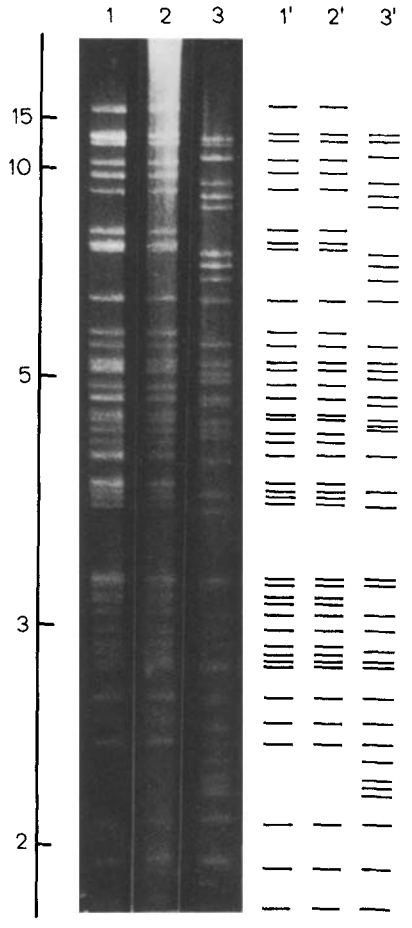

Fig. 2. Restriction endonuclease patterns of mtDNA from *Nicotiana sylvestris* (lanes 1 and 1′) *N. tabacum* (lanes 2 and 2′) and Line-92 (lanes 3 and 3′) after digestion with *Xho*I. Mitochondria were isolated from green leaves. Lanes 1′, 2′ and 3′ are diagrammatic presentations of the bands in lanes 1, 2 and 3, respectively. Molecular weights were estimated as in Fig. 1

was performed lengthwise on horizontal 27.0 cm × 14.0 cm × 0.4 cm slab gels containing 0.7% agarose. Samples of 50 µl containing 1–2 µg mtDNA were loaded at each position. Electrophoresis proceeded for 42–48 h (2–3 V/cm, room temperature) in running buffer (40 mM Tris-acetate, 20 mM sodium acetate, 1 mM EDTA, pH 7.8).

After electrophoresis the gels were stained with ethidium bromide (1 µg/ml, 30 min or longer) in running buffer, briefly washed and photographed with 300 nm ultraviolet light.

Results

Reference Plants

We focussed our attention on the following four plant types: *N. tabacum*, *N. sylvestris*, Line-92 and Type A. These types were either the direct progenitors (e.g. Type A and *N. tabacum*) of the tested cybrids or were the somatic parents (e.g. *N. sylvestris* and Line-92) of one of the direct cybrid progenitors (Type A) which was itself a cybrid (Zelcer et al. 1978). Plastid-DNA restriction patterns and other chloroplast markers (see Aviv et al. 1980) failed to indicate any differences between *N. tabacum* and *N. sylvestris* or between Type A and Line-92. However, *N. tabacum* (or *N. sylvestris*) chloroplasts clearly differed from chloroplasts of Line-92 (or Type A). Figure 1 shows restriction endonuclease digestion patterns of *N. tabacum* and Line-92 mtDNAs. The data indicate that these two plant types also differ with respect to their mtDNA restriction patterns (*Xho*I and *Sal*I). A comparison (Fig. 2) of *N. sylvestris* and *N. tabacum* mtDNAs, on the other hand, showed that these two species had indistinguishable *Xho*I restriction patterns but both clearly differed from Line-92. The restriction patterns obtained following digestion with *Sal*I (not shown) led to a similar conclusion. Likewise, when Type A mtDNA was compared with *N. tabacum* and Line-92 mtDNAs (Fig. 3), the *Xho*I restriction patterns of Type A and *N. tabacum* differed while those of Type A and Line-92 were indistinguishable with respect to all but one fragment. The close similarity (22 out of 28 bands) between Line-92 and Type A mtDNAs was also revealed after *Sal*I digestion (not shown).

The molecular contour lengths of mtDNA from *Nicotiana* and other higher plants fall considerably shorter than the sum total of restriction endonuclease fragment lengths found for these same DNAs (Sparks and Dale 1980; Edelman 1981). Technically it is more convenient to isolate mtDNA from cultured cells than from green plant tissue. Moreover, cell suspensions have a great advantage in *Nicotiana* where en masse production of

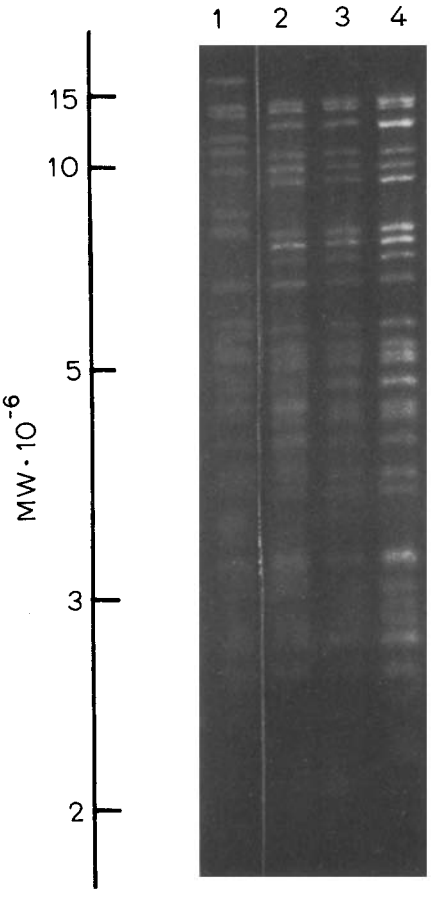

Fig. 3. Restriction endonuclease patterns of mtDNA from *Nicotiana tabacum* (lane 1), Type A (lane 2), and Line-92 (lanes 3 and 4), after digestion with *Xho*I. Mitochondria were isolated from green leaves (lanes 1, 2 and 3) or from cell suspensions (lane 4). Molecular weights were estimated, as in Fig. 1

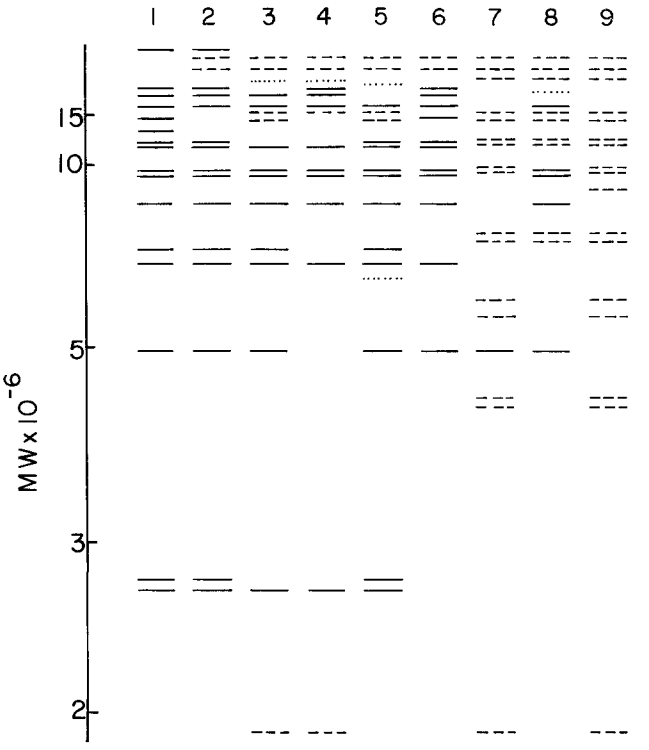

Fig. 4. Schematic diagram of mtDNA restriction patterns of parental plants and cybrids after digestion with *Sal*I. Solid and dashed lines mark the fragments unique to the fertile parent (*N. tabacum*) and sterile parent (Type A) respectively. Fragments common to both parental plants are not shown. Fragments present in a cybrid which do not exist in a parental type are marked by dotted lines. Lane 1, fertile parent (*N. tabacum*). Lanes 2, 3 and 4, fertile cybrids (B-13-4, A-10-5 and A-10-7, respectively) having *N. tabacum* chloroplasts. Lanes 5 and 6, fertile cybrids (C-12-2 and A-45-3, respectively) having Line-92 chloroplasts. (Fragment smaller than 4×10^6 daltons were not resolved in the gel for lane 6.) Lane 7, sterile cybrid (D-34-2) having *N. tabacum* chloroplasts. Lane 8, sterile cybrid (A-6-3) having Line-92 chloroplasts. Lane 9, sterile parent (Type A). Molecular weight estimations as in Fig. 1

etiolated seedlings is not possible. It was therefore important to determine whether cultured cell mtDNA differed in its restriction patterns from green leaf mtDNA of the same type. In all comparisons from several plant types the restriction patterns were identical. Figure 3 shows an example of such a comparison. Thus, wherever applicable, cell suspension cultures rather than intact plant tissue were used as representative sources of mtDNA in this study.

Cybrid Plants

Four types of cybrids resulting from the fusion between Type A (recipient) and *N. tabacum* (donor) protoplasts were tested. With respect to cytoplasmic characteristics, the recipient plants were *L-92/ster* (i.e. having Line-92 chloroplasts and being male sterile) while the donor plants were *tab/fert* (i.e. having *N. tabacum* chloroplasts [=*N. sylvestris* chloroplasts] and being male fertile). All the cybrids had Type A (i.e. *N. sylvestris*) nuclei and chloroplast/anther characters of either of the parental plants, or novel compositions (*tab/ster* and L-92/*fert*). Representative plants of the four cybrid types were taken for mtDNA analysis. Figures 4 and 5 summarize the results by comparing the cybrid mtDNA restriction patterns obtained after digestion with *Sal*I and *Xho*I, respectively, with the mtDNA restriction patterns of donor-and recipient parental plants. To simplify analysis and presentation of data, fragments common to both parents were eliminated from the Figures. The results indicate that after digestion with either restriction endonuclease all sterile, cybrid mtDNA restriction patterns were similar to the respective pattern of the sterile recipient parent while all fertile cybrids patterns were similar to the respective pattern of the fertile donor parent. It should be noted that in all but one cybrid, new DNA fragments, not detected in either the recipient or the donor parent, were revealed.

Independent Assortment of Chloroplasts and Mitochondria in Cybrid Plants

The chloroplast character of the cybrids was previously defined mainly by sensitivity or resistance to tentoxin (Aviv and Galun 1980). In the present study we analyzed the plastid DNA restriction pattern of several cybrids. All analyses confirmed the chloroplast characters reported previously (Aviv and Galun 1980) and showed no correlation between anther character and chloroplast type in individual cybrids. The restriction patterns of two such cybrids, both with restored fertility but one (A-10-7) tentoxin resistant (as the donor parent) and the other (A-45-3) tentoxin sensitive (as the recipient parent), are presented in Fig. 6. The results indicate that A-10-7, shown above (Fig. 4 and 5) to con-

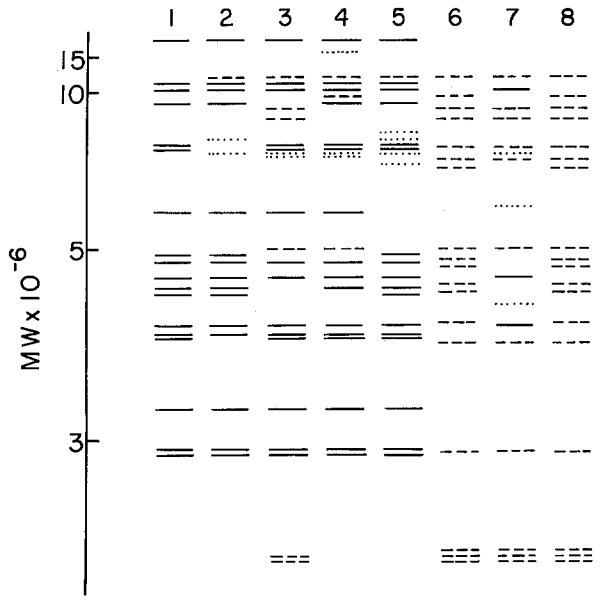

Fig. 5. Schematic diagram of mtDNA restriction patterns of parental plants and cybrids after digestion with *Xho*I. Solid, dashed and dotted-line fragment designations are as in Fig. 4. Lane 1, fertile parent (*N. tabacum*) Lanes 2, 3 and 4, fertile cybrids (B-13-4, A-10-5 and A-10-7, respectively) having *N. tabacum* chloroplasts. Lane 5, fertile cybrid (C-12-2) having Line-92 chloroplasts. Lane 6, sterile cybrid (D-34-2) having *N. tabacum* chloroplasts. Lane 7, sterile cybrid (A-6-3) having Line-92 chloroplasts. Lane 8, sterile parent (Type A). Molecular weight estimations as in Fig. 1

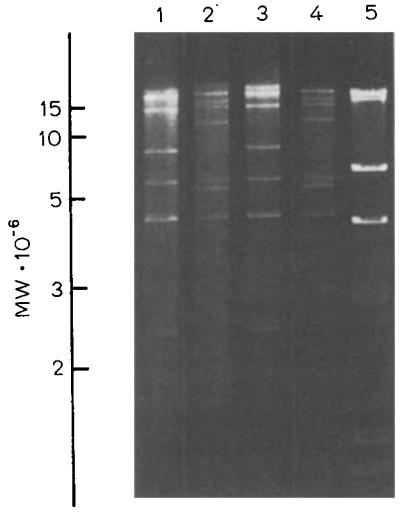

Fig. 6. Restriction endonuclease analysis of chloroplast DNA from *Nicotiana tabacum* (lane 1), cybrid A-43-3 (lane 2), cybrid A-10-7 (lane 3) and Line-92 (lane 4) after digestion with *Bgl*I. Lane 5 is a *Hin*dIII digest of λ phage DNA used as molecular marker

tain mtDNA similar to *N. sylvestris* (=*N. tabacum*), had the plastid DNA restriction pattern (*Bgl*I) of *N. tabacum* while cybrid A-45-3, which also contained mtDNA similar to *N. sylvestris*, had the plastid DNA pattern of Line-92 type. Taken together, these results indicate that in cybrids A-45-3, C 12-2 and D-34-2 independent assortment of mitochondria and chloroplasts has occurred.

Discussion

The results reported in this paper (Figs. 1, 2, and 3) suggest that mtDNA restriction patterns of the parental plants, Type A and *N. tabacum*, differ sufficiently one from another to enable comparisons with mtDNAs from individual cybrid progeny plants. The close similarity between the mtDNA restriction patterns of *N. tabacum* and *N. sylvestris* (Fig. 2) despite their long evolutionary separation is noted also (see Smith 1975).

Mitochondrial DNA restriction analyses of cybrids with either Type A (i.e. Line-92) or *N. tabacum* chloroplasts (Figs. 4 and 5) suggested that cytoplasmic hybridization between plants differing in both mitochondria and chloroplasts can result in independent assortment of these organelles in the cybrid progeny. This finding, which is of significance to future study of organelle genetics in angiosperms, was not previously expressed but conforms to other results obtained by plant protoplast fusion. Thus, Scowcroft and Larkin (1981) reported random assortment of nuclei and chloroplasts in somatic hybrids resulting from fusion between protoplasts of two variants of *N. debneyi* which differed in nuclear and chloroplast markers; however assortment of mitochondria was not followed in this study. Iwai et al. (1980) fused *N. tabacum* and *N. rustica* protoplasts and analysed the fraction 1 protein (ribulose bisphosphate carboxylase) in the somatic hybrids. Independent assortment of the small and the large subunits was revealed, indicating independent assortment of nuclei and chloroplasts; however no data on mitochondria were presented. Independent assortment of nuclei and mitochondria was indicated in somatitc cybrids resulting from fusing *N. tabacum* and *N. debneyi* cytoplasms (Belliard et al. 1979). Moreover, integration of the data from the latter report with those of Belliard et al. (1978) intimated independent assortment of nuclei, chloroplasts and mitochondria. While the above-cited reports focussed on either chloroplasts or mitochondria, they are in harmony with our evidence showing independent assortment of chloroplasts and mitochondria from heteroplasmons obtained by protoplast fusion.

Our results indicate also that a correlation exists between anther fertility and mtDNA restriction patterns: the restriction patterns of cybrids with viable pollen were similar to the respective pattern of the fertile parent while the mtDNA restriction patterns of the cybrids lacking viable pollen were similar or identical to the respective pattern of the sterile parent. This correlation was observed when the mtDNA was fragmented with either *Xho*I or *Sal*I. Although the mtDNA restriction patterns varied within each of the two cybrid groupings, several fragments consistantly correlated with either the fertile or the sterile parents (Table 2 and 3). No correlations were found between sterility/fertility and chloroplast DNA (Fig. 6).

Figures 4 and 5 show the existence in cybrids of new fragments not found in either parental mtDNA restriction pattern. The restriction patterns of individual cybrids did not change in subsequent generations [e.g. the second and third generations of cybrids f-29 and f-123 were checked (data not shown)]; also the total number of mtDNA fragments in the cybrids was similar to that in the parents. Thus it is possible that the mtDNA restriction patterns of the cybrids might represent new populations. Our data agree with the interpretation of Belliard et al. (1979) who found variations in somatic hybrid mtDNA restriction patterns and attributed them to mitochondrial DNA recombinations. A correlation was also noted between cytoplasmic male sterility/fertility and the mtDNA restriction pattern (but not the chloroplast DNA pattern [Belliard et al. 1978]). Both the *N. tabacum*/*N. debneyi* system, used by Belliard et al. (1979)

Table 2. Correlation between *Sal*I mtDNA fragments and anther sterility/fertility in parental types and in cybrids resulting from fusion between X-irradiated *N. tabacum* (fertile) protoplasts and non irradiated protoplasts of Type A (sterile) plants

Fragment molecular weight ($\times 10^{-6}$)	Parental plants				Cybrid progeny							
	N. tabacum and *N. sylvestris*	Line 92	Type A		Steriles		Fertiles					
			f-48	f-29[a]	A-6-3	D-34-2	3-13-4	A-10-5	A-10-7	C-12-2	A-45-3	f-123[b]
11.3	−	+	+	+	+	+	−	−	−	−	−	−
11.0	−	+	+	+	+	+	−	−	−	−	−	−
6.6	+	−	−	−	−	−	+	+	+	+	+	+
2.6	+	−	−	−	−	−	+	+	+	+	?[c]	+

[a] Type A plant used in a fusion experiment not detailed in this report
[b] Fertile cybrid obtained after fusion between f-29 (Type A) and X-irradiated *N. tabacum* protoplasts
[c] Not determined

Table 3. Correlation between *Xho*I mtDNA fragments and anther sterility/fertility in parental types and in cybrids resulting from fusion between X-irradiated *N. tabacum* (fertile) protoplasts and non-irradiated protoplasts of Type A (sterile) plants

Fragment molecular weight ($\times 10^{-6}$)	Parental types				Cybrid progeny						
	N. tabacum and *N. sylvestris*	Line 92	Type A		Steriles		Fertiles				
			f-48	f-29[a]	A-6-3	D-34-2	B-13-4	A-10-5	A-10-7	C-12-2	f-123[b]
10.6	+	−	−	−	−	−	+	+	+	+	+
4.70	+	−	−	−	−	−	+	+	+	+	+
3.65	−	+	+	+	+	+	−	−	−	−	−
3.10	+	−	−	−	−	−	+	+	+	+	+
2.80	−	+	+	+	+	+	−	−	−	−	−
2.77	+	−	−	−	−	−	+	+	+	+	+
2.74	+	−	−	−	−	−	+	+	+	+	+
2.20	−	+	+	+	+	+	−	−	−	−	−

[a] Type A plant used in a fusion experiment not detailed in this report
[b] Fertile cybrid obtained after fusion between f-29 (Type A) and X-irradiated *N. tabacum* protoplasts

and our *N. tabacum*/Line-92 system are of the alloplasmic male sterility type (Gerstel 1980). As pointed out by Scowcroft (1979), differences in chloroplast compositions between male-sterile and male-fertile lines, as reported by Frankel et al. (1979) for *Nicotiana* and by Chen and Meyer (1979) for *Gossypium*, need not indicate a causal relationship between plastome and male-sterility.

Mitochondria have been correlated with male sterility in maize (Miller and Koeppe 1971) and wheat (Quetier and Vedel 1977). Further more, there is evidence for the participation of mitochondria in the genetic regulation of male sterility in maize (see: Levings and Pring 1979; Forde and Leaver 1980). However in wheat and maize somatic hybridization is not yet technically possible hence chloroplasts and mitochondria are transmitted (maternally) together during sexual propagation. As a result the role of chloroplasts in the control of male-sterility is not easily assessed in these plants. The cytogenes controlling male sterility may reside in cytoplasmic organelles as molecules physically separated from the rest of the organelle DNA. The existence of distinct species of small (1.4–6.2 kilobases) DNA molecules in mitochondria of male-fertile but not in male-sterile maize cytoplasms is evidence for this (Kemble et al. 1980). The exact location of DNA sequences controlling cytoplasmic male-sterility in angiosperms requires therefore further study, in which cytoplasmic hybridization – of the kind reported by us – can serve as one of the tools.

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