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Variant mitochondrial plasmids of broad bean arose by recombination and are controlled by the nuclear genome

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ABSTRACT

Various cytoplasms of broad bean contain three mitochondrial plasmids (mtp1, 2 and 3), previously described. In cytoplasm 350 we have observed several additional mitochondrial plasmids, varying in number and in identity according to the nuclear background. Replacement of the nucleus by backcrossing led to the appearance or disappearance of additional plasmids, indicating that the nuclear genome controls either the creation or the copy level of mitochondrial plasmids. Analysis of eight variant additional plasmids (mtp4–11) suggests that they all result from a double recombination event between mtp1 and mtp2. In all cases, one recombination point was located within a 276-bp sequence, identical in both plasmids. For 7 plasmids, the region in which the second recombination event occurred could be narrowed down to a short stretch containing imperfect tandem repeats of a 31-bp motif. The largest sequence shared by the recombination regions was hexanucleotide GCGACG.

INTRODUCTION

Mitochondria of several plant species contain small (< 5 kb), circular plasmids (for a review, see ref. 1). The sequences of these plasmids, even in related species, are not very similar. Many if not all are transcribed but no gene product has ever been detected and no significant open reading frame ever identified. However, RNA editing of plant mitochondrial transcripts (2–4) could create open reading frames that are not obvious from the DNA sequence. In most instances, more than one mitochondrial plasmid has been identified in the same species. In such cases, the plasmids may share some sequences, but most often identity is not very high. So far the role, if any, of these plasmids remains totally elusive. Unfortunately, their uniparental (maternal) inheritance and the absence of a transformation system for the plant mitochondrial genome have restricted investigations to descriptive approaches.

In broad bean (*Vicia faba* L.), three supercoiled plasmids, mtp1 (1704 bp), mtp2 (1695 bp) and mtp3 (1478 bp), have been described in fertile (5,6) as well as male sterile (7) lines. They all are transcribed (7) and replicative forms have been observed by electron microscopy (8). Plasmids mtp1 and mtp2 share more than 80% homology along two-thirds of their sequences but the region transcribed is the non-homologous portion.

Besides mtp1, 2 and 3, an additional plasmid has been observed in the line G58 bearing the male sterile cytoplasm 350. Cloning and restriction analysis have revealed a close relationship between this plasmid and mtp1 (9). Here, we have analyzed this and seven other additional plasmids in more detail. They all seem to result from recombination events specifically occurring in two regions of mtp1 and mtp2. We further report observations suggesting that the presence of additional plasmids could be controlled by the nuclear genome.

MATERIALS AND METHODS

Plant material

Cytoplasm 350 induces male sterility when combined with a maintaining nuclear genome (10). Fertile genotypes 196, G58 and 127 are nearly pure lines selected from two German populations (127 is issued from cv. Fribo and G58 from cv. Minuta) and from a Belgian population (196 is issued from cv. Maxime). These genotypes were fixed by 7 (127 and 196) or 12 (G58) generations of selfing. Their isogenic male sterile forms were obtained by 9 (196 and 127) or 13 (G58) backcrosses on cytoplasm 350. These lines as well as wild representatives and cultivars belong to the INRA collection (Dijon).

DNA preparation

Mitochondrial DNA from pooled plants was prepared from DNAse-treated mitochondria (to eliminate contaminant DNA) and purified by isopycnic centrifugations as described (11).

Mitochondrial DNA from individual plants was prepared as follows. Leaf tissues (0.5–5 g, fresh weight) were ground with a mortar and pestle in the presence of 10 ml homogenization buffer (0.4 M sucrose, 50 mM Tris, 10 mM KH_2PO_4, 1 mM EGTA, 5 mM β-mercaptoethanol, 1% BSA, pH 7.6 (HCl)) and 1 g glass beads (0.25–0.30 mm in diameter). The homogenate

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was filtered through 3 Miracloth layers and centrifuged first for 10 s at 5,000 rpm (Sorvall SS34), then for 10 min at 15,000 rpm (Sorvall SS34). The pellet was resuspended in 1 ml (for 5 g fresh weight) of suspension medium (0.3 M mannitol, 10 mM K_2HPO_4, 0.15 M NaCl, 1 mM EDTA, 0.1% BSA, pH 7.2 (KOH)) supplemented with 10 mM $MgCl_2$ and 50 µg DNase I. After 30 min at 37°C, the mitochondria were collected by centrifugation. The pellet was lysed for 30 min at 56°C in 400 µl of 2% Sarkosyl and 10 mg/ml proteinase K. After phenol, phenol–chloroform (1:1) and ether extraction, nucleic acids were precipitated with ethanol.

MtDNA library

MtDNA digested with *Pvu*II or *Eco*RI was respectively cloned into the *Sma*I or *Eco*RI site of bacterial plasmid pTZ (USB Research). Clones containing derivatives of mtp1 or mtp2 were selected by hybridization with mtp6 DNA prepared from a bacterial clone.

Southern blot hybridization

MtDNA was cut by restriction endonucleases in the presence of RNase, electrophoresed in an agarose gel, and transferred by capillarity to a Hybond-N membrane (Amersham). Prehybridization was carried out for 5 h at 42°C in 50% formamide, 5×SSC, 5×Denhardt's, 1% SDS, 250 µg/ml salmon sperm DNA. Hybridization was carried out for 20 h in the same medium supplemented with the nick-translation-labeled probe (± 10^6 cpm/ml). Membranes were washed three times for 5 min in 2×SSC, 0.1% SDS at room temperature, for 15 min in 2×SSC, 0.1% SDS at 50°C, and for 15 min in 0.5×SSC, 0.1% SDS at 50°C.

DNA sequencing

DNA was sequenced from single-stranded DNA by the dideoxy method (12). Primers were 14 to 16-mer oligonucleotides scattered through the mt plasmids. The entire sequences of plasmids mtp4, 5 and 6 were determined on both strands. Plasmids mtp7 through 11 were identified by restriction and sequenced only in the region encompassing the recombination points, i.e. between the two following primers : TTCCTGAG-ATAACCG (1439–1453 of mtp4) and TCCCGAAACCTCATA (antisense of 124–138 of mtp4).

RESULTS

The presence of an additional plasmid in the G58 line bearing the male sterile cytoplasm 350 (9) led us to investigate other lines associating either a fertile (N) or a sterile (350) cytoplasm with 3 different nuclear backgrounds (196, G58 and 127). We focused our analysis on mtp1 (1704 bp), mtp2 (1696 bp) and their derivatives, a preliminary restriction analysis (not shown) of additional plasmids having suggested that they contain sequences from both mtp1 and mtp2. The structures of the latter plasmids have been previously described (6,7) and are schematized in Fig. 1. Two-thirds of their sequences are homologous. In the homologous region, sequence identity exceeds 50% and two sectors are 100% identical (white sectors in Fig. 1). Two particular structural features are found in the homologous regions. One consists of 8 (mtp1) or 6 (mtp2) direct repeats of a 31 bp motif (arrows in Fig. 1), the other of six inverted repeats liable to fold into hairpin structures (double arrows in Fig. 1).

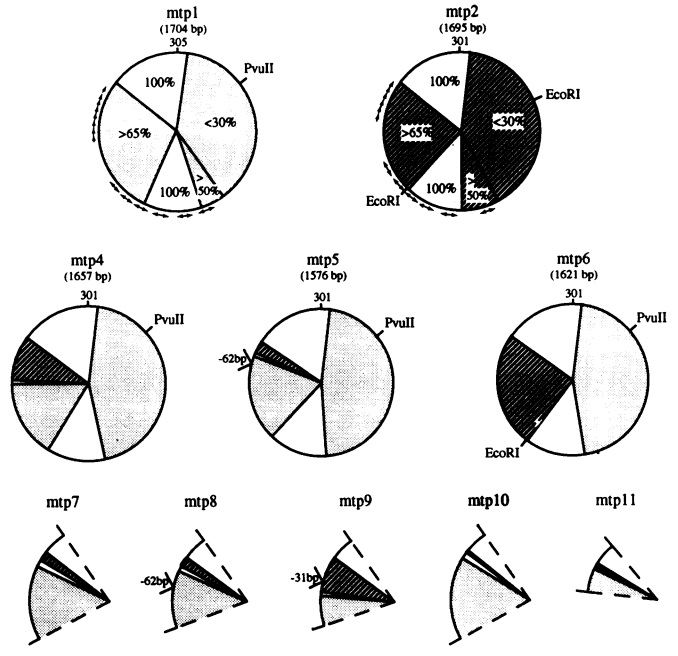

Figure 1. Structure of the variant plasmids isolated from various lines. The structure of mt plasmids 1 through 11 is deduced from their sequences (ref. 7 for mtp1,2; code Genbank L21659–21665 for mtp4-11). Regions specific to mtp1 are stippled. Regions specific to mtp2 are hatched. Regions whose origin could not be assigned to either mtp1 or mtp2 because the plasmids are totally homologous there, are in white. Inverted (→ ←) and direct (→) repeats are indicated only for mtp1 and mtp2. Deletions (−62 bp, −31bp) are indicated.

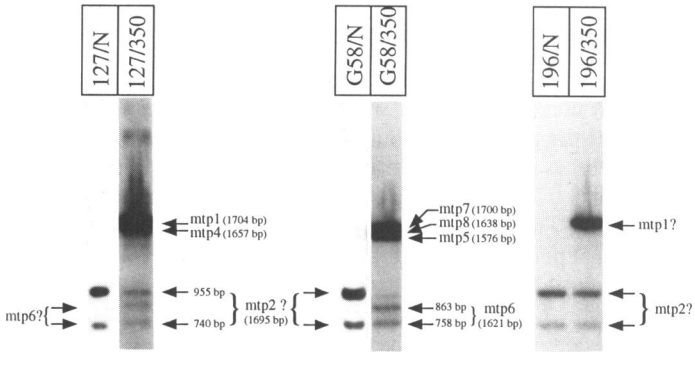

Figure 2. Mitochondrial plasmid distribution in a fertile (N) and a sterile (350) cytoplasm associated with three nuclear backgrounds (196, G58 and 127). MtDNA was prepared from pooled etiolated seedlings (50 g), cut with *Pvu*II and *Eco*RI, electrophoresed on agarose gel (1.2%), transferred to a nylon membrane, and hybridized with ^{32}P-labeled mtp6. Plasmid identities and sizes are indicated for the lines from which they were cloned and sequenced (see Fig. 3, Fig. 4 and Table 1) except for mtp2 which was identified previously (ref. 7). In the other cases, identification is followed by a question mark since it is based only on size.

Identification of additional plasmids in various lines

Mitochondrial DNA of the lines above mentioned was cut with *Pvu*II and *Eco*RI. While mtp1 is cut once by *Pvu*II, giving rise to a 1704-bp fragment, mtp2 is cut twice by *Eco*RI, giving rise to two fragments 955 and 740 bp long. The DNA was then electrophoresed and hybridized with mtp6. This additional plasmid possesses sequences of both mtp1 and mtp2 (see below).

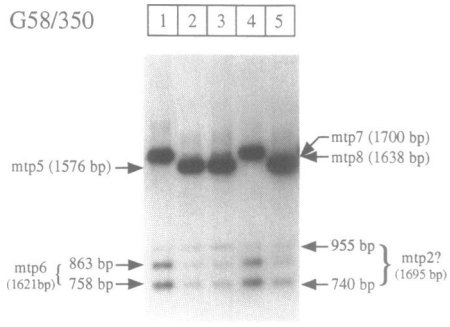

Figure 3. Polymorphism of mitochondrial plasmids within the G58/350 line. Mitochondrial DNA from five individual plants of the G58/350 line was characterized as in Fig. 2. Identity and size of mtp6, mtp7 and mtp8 refer to plant 4. Plasmids mtp2 and 5 are identified in Fig. 2.

It is a convenient probe for revealing both of these as well as additional plasmids. When cytoplasm N (fertile) was associated with any of three different nuclear backgrounds (127, G58 and 196), only mtp2 appeared (Fig. 2). However, analysis of sterile cytoplasm 350 revealed the presence of additional plasmids (mtp4−11) which seemed to differ according to the nuclear background.

To facilitate the comprehension of the Southern blot (Fig. 2), we have designated the various additional bands according to their exact size, although this information derives from the cloning and sequencing steps to be described later. In addition, plasmids are formally named only in those lines from which they were cloned and sequenced. In the other cases, identification is followed by a question mark, since it is based only on size.

Mt plasmid polymorphism within a line

In two cases (127/350 and G58/350) a broad hybridization band (Fig. 2) corresponds with several plasmids of slighly different sizes, as the results of cloning and sequencing will show. Since these observations were made on DNA prepared from a pool of plants, it was interesting to check the homogeneity of this material. Therefore, we hybridized DNA prepared from individual plants of the G58/350 genotype (Fig. 3). We observed band segregation in the upper part of the gel. One band is presumably mtp5 cloned from the pool of plants (Fig. 2). Another band is compatible with the size of mtp1 identified in 127/350 (Fig. 2), but will be shown later to resolve into two new plasmids (mtp7 and mtp8). This plasmid polymorphism was confirmed in 16 other individual plants (results not shown).

Mt plasmids vary with the nucleus

The presence of different plasmids in a same cytoplasm associated with different nuclear backgrounds suggests that the nucleus somehow controls the creation or level of mt plasmids. The lines with the 350 cytoplasm appearing in Fig. 2 resulted from 9−13 backcrosses between the source of the 350 cytoplasm and the G58, 196 and 127 nuclei associated with a normal cytoplasm. We also possessed a fourth backcross between the various lines analyzed in Fig. 2 and could thus monitor at an earlier stage and in individual sister plants the fate of plasmids of the 350 cytoplasm transferred, e.g. from 127 to G58. Four sister plants of the fourth backcross between 127/350 (female parent) and G58/N (male parent) were individually analyzed as previously (Fig. 4A).

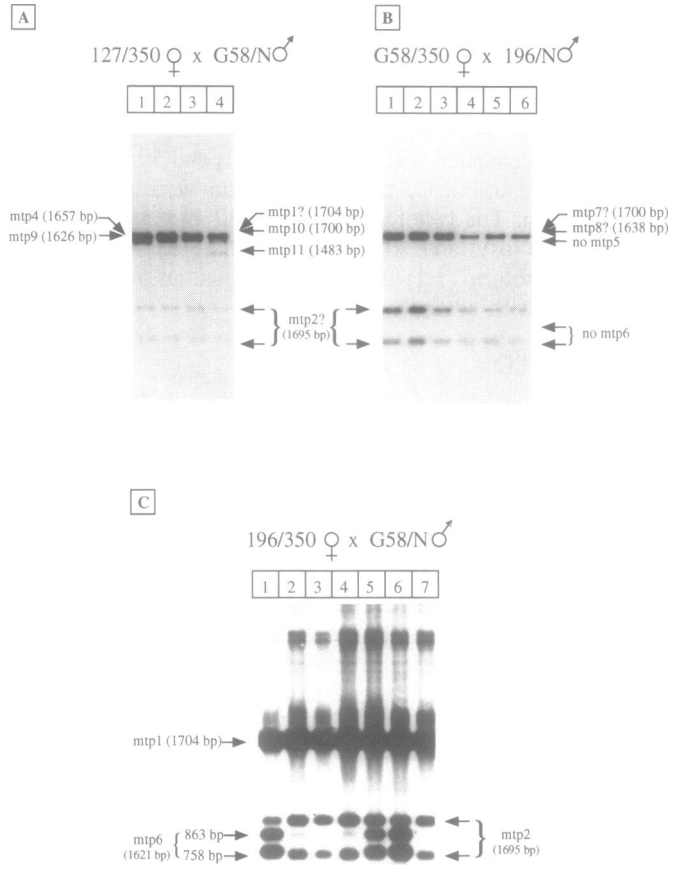

Figure 4. Modification of variant mitochondrial plasmids after backcrossing with various nuclear genomes. Mitochondrial DNA from individual plants resulting from four backcrosses of the indicated lines was analyzed as in Fig. 2. **A:** Fourth backcross of 127/350 by G58/N. DNA from plant 4 was used to clone mtp4, 9, 10 and 11. **B:** Fourth backcross of G58/350 by 196/N. **C:** Fourth backcross of 196/350 by G58/N. DNA from plant 1 was used for mtp1 and mtp6 cloning and identification.

Unlike the female parent, none of the four plants contained any mtp6-like plasmid. The broad band seen at ~1700 bp when several plants were pooled (Fig. 2) resolved here into several bands (mtp4, 9, 10), the ratio between them differing from plant to plant. Moreover, plant 4 contained an additional band (~1483 bp, mtp11).

From the fourth backcross of G58/350 with 196/N, analysis of 6 sister plants is displayed. Plasmids mtp5 and mtp6, originally present in G58/350, were not detected in any of them (Fig. 4B) neither in a pool of 10 other plants (not shown). In the reciprocal cross 196/350×G58/N, 7 sister plants resulting from the fourth backcross were analyzed (Fig. 4C). Five of them contain an mtp6-like band that was faint in two plants (2 and 4) and normally intense in three other plants (1, 5 and 6). No such signal was detected in the plants 3 and 7. In this overexposed autoradiogram, bands of higher molecular weight appear, possibly corresponding with open-circular plasmid forms undigested by the restriction enzymes. However, we cannot exclude that they represent plasmids or mtDNA unidentified so far and present in low amount in the mitochondrial genome.

Table 1. Genotypic origin of mt plasmids

Plasmids	Size (bp)	Nucleus	Number of clones characterized by restriction	sequencing
mtp1	1704	127	3	1
		196×G58	26	8
mtp2	1695	196×G58	4	4
mtp4	1657	127×G58	3	3
mtp5	1576	G58	1	1
mtp6	1621	196×G58	14	14
		G58	6	6
mtp7	1700	G58	13	6
mtp8	1638	G58	2	2
mtp9	1626	127×G58	3	2
mtp10	1700	127×G58	9	3
mtp11	1483	127×G58	1	1

Nucleus indicates the nuclear background (associated with cytoplasm 350) of the plants from which plasmids were isolated. 196×G58 and 127×G58 represent the fourth backcross of the first by the second genotype. Mtp1 and mtp6 were identified in two different nuclear backgrounds.

Additional plasmids arose by recombination between mtp1 and mtp2

We have thus identified 8 variant plasmids in cytoplasm 350 associated with various nuclei. Preliminary analysis (not shown) indicated that the additional plasmids contain the *Pvu*II site of mtp1. To further elucidate their molecular structure, we cut mtDNA from several plants with *Pvu*II and cloned the fragments in a bacterial plasmid. Clones containing sequences homologous to mtp1 or mtp2 were isolated and further analyzed. Their origin is reported in Table 1.

Mtp4 (1657 bp), mtp5 (1576 bp) and mtp6 (1621 bp) were completely sequenced. Their structures (Fig. 1) clearly show that they arose from recombination between mtp1 and mtp2: each plasmid can be divided into two regions totally identical to either mtp1 (stippled sectors) or mtp2 (hatched sectors). The other five additional plasmids were sequenced only in the region where recombination between mtp1 and mtp2 presumably occurred. Recombination points could not be precisely assigned because they belong to regions where mtp1 and mtp2 are identical (white sectors). One of these regions (276 bp) is the large, identical region (white sector) downstream of the direct repeats. For seven plasmids, the second recombination event was located in a region characterized by direct repeats of a 31-bp motif. However, these repeats display some heterogeneity within a plasmid and between mtp1 and mtp2 (Fig. 5A), so that the recombination points could be confined to a short sequence. Each recombinant plasmid thus possesses a unique series of repeats, some derived from mtp1 and others from mtp2. In agreement with the size variation of these plasmids, the number of repeats was found to vary (Fig. 5B). This could be due to recombination occurring between different parental units and, in mt plasmids 5, 8 and 9, to internal deletions of one or more repeats before or after recombination. The deletions, which exactly correspond with 1 (mtp9) or 2 (mtp5 and 8) repeats, are not cloning artefacts, since either the plasmid sizes revealed by Southern blot analysis of mt DNA corresponded with the calculated molecular weights or several independent clones were found to be similar. Deletions could arise from recombination between two identical molecules or from slipped mispairing or copy choice during DNA replication (13). For each plasmid, the stretch where recombination must have occurred was determined as narrowly as possible. When these sequences

A

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mtp1
1          GCGACGGAGGAGCAATTCTGATTA
2    TAAGCTT------T--------GGAA-----
3    ----------------------G-A------
4    ----------------------G-TC-C--T
5    -T----A---------------G--------
6    ----------------------G-TC-C--T
7    -T----A------T------T-GGAA----C
8    ----------------------G-AGT-A-

mtp2
1'         ---------------G-AGT-AA-CCAG
2'   ----------------------G--AT----
3'   ----------------------G-A------
4'   ----------------------G-ACTC--CTCTTATTCGTTGAGCTTAC
5'   ------A------T------T-G-ACT-AG-
6'   ------A-------------T-G-TCT
```

B

mtp	Repeat series	Recombination sequence
mtp1	-1-2-3-4-5-6-7-8-	
mtp2	-1'-2'-3'-4'-5'-6'-	
mtp4	-1-2/2'-3'-4'-5'-6'-	TAAGCTTGCGACG
mtp5	-1-4-5/5'-6'-	AGCTAGCGACG
		del TGATTATAAGCTTGCGACG
mtp7	-1-2-3-4-5-6-7/5'-6'-	AGCTAGCGACGTAGGAGCTAG
mtp8	-1-2-5-6-7/5'-6'-	AGCTAGCGACGTAGGAGCTAG
		del GCGACGGAGGAGCAAGT
mtp9	-1-2/2'-4'-5'-6'-	TAAGCTTGCGACG
		del ATTATAAGCTTGCGACGGAGGAGCAAGTA
mtp10	-1-2-3-4-5-6-7-8*/6'-	TAAGCTAGCGACGGAGGAGC
mtp11	-1/6'-	GCGACGGAGGAGC

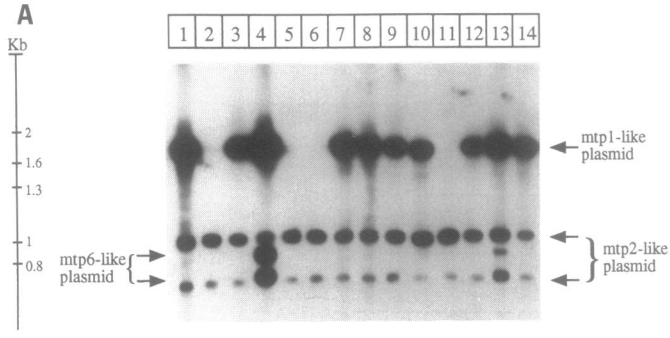
Figure 5. Tandem repeats of mt plasmids. **A:** The 8 repeats of mtp1 and the 6 repeats of mtp2 are aligned. Dashes indicate conserved residues. **B:** The succession of repeat units, as determined by sequencing, is depicted for variant plasmids. The last column displays the sequence where recombination occurred, determined as narrowly as possible. It is the sequence shared by the mtp1 repeat and the mtp2 repeat which presumably participated in the recombination. For mtp5, mtp8 and mtp9, internal one- or two-unit deletions defined a recombination region which is displayed (del). For mtp10, 8* means that unit 8 has a single base mutation (T→A) compared to unit 8 of mtp1. The GCGACG sequence found in all cases is underlined.

were compared, all were found to share the hexanucleotide GCGACG (Fig. 5B). Plasmid mtp6 was an exception, its second recombination point being located in a 204-bp stretch identical in mtp1 and mtp2 and belonging to a region of inverted repeats.

Additional plasmids in wild varieties and cultivars

We identified additional plasmids in cytoplasm 350 associated with some nuclear genotypes but not in the N (fertile) cytoplasm (Fig. 2) or in the other male sterile cytoplasm (447) (data not shown). To broaden this observation, we analyzed a series of individual plants belonging to 38 wild varieties and 19 cultivars of broad bean to determine whether mitochondrial plasmids were present. We excluded from our analysis mtp3, previously shown

A



B

Group I mtp2-like plasmid	Group II mtp1- and mtp2-like plasmids		Group III mtp1-, mtp2- and mtp6-like plasmids
Bourdon	Côte d'Or	Yemen 1288	Survoy
Blandine	Ascott	Afghanistan 775	Spain 335 (13)
Wieboon	Troy	Ecuador 1442 (7)	China 390 (4)
Bulldog	Maris-bead	Japan 351 (3)	
Optica	Arla	Egypt 338	
Avrissot	Kristall	Hungary 617	
Alfred	Cagnotte	Yugoslavia 811	
Minica	Excelle	Italy 781	
India 778 (11)	Diana	Ethiopia 317	
Japan 349 (2)	Pilote	Afghanistan 318	
Iran 363	USSR 320	Italy 615	
China 393 (5)	Marocco 336 (10)	Iran 368	
China 1450 (6)	Nepal	Sudan 337	
	USSR 799	Bolivia 845 (9)	
	Cyprus 1370 (12)	Syria 766	
	Greece 362	Poland 321	
	Hungary 614	Peru 886 (1)	
	USSR 319 (14)	Ecuador 862	
	Turkey 829	Ecuador 1441 (8)	
	Portugal	Sweden 837	
	Nepal 387		

Figure 6. Identification of mitochondrial plasmids in wild representatives and cultivars of broad bean. Mitochondrial DNA from individual plants of 38 wild representatives and 19 cultivars of broad bean was analyzed as in Fig. 1. Panel A displays the results obtained for 12 wild representatives. In panel B, the 57 genotypes were classified as follows: Group I contains genotypes for which only the two bands characterizing mtp2 were detected. Group II includes those genotypes which produced mtp2- and mtp1-like hybridization signals. Group III includes those genotypes which displayed mtp1-, mtp2- and mtp6-like hybridization signals. Numbers in brackets refer to the genotypes displayed in panel A.

to be present in all lines analyzed and whose sequence differs completely from that of mtp1 and mtp2. MtDNA was thus cut with *Pvu*II and *Eco*RI and hybridized with mtp6. Fig. 6A gives an example of the results observed. The majority (41) of genotypes had both mtp1- and mtp2-like plasmids (Fig. 6B). Three displayed an additional band of approximatively 850 bp which could belong to an mtp6-like plasmid. Finally, 13 genotypes had only an mtp2-like plasmid. We extended our analysis to mtDNA prepared from other species of the genus *Vicia*. Hybridization signals were obtained for *Vicia narbonensis*, thought to be the ancestor of *Vicia faba* (14), but not for more distant species such as *V. galilea, V. hyaeniscyamus* or *V. melanops* (results not shown).

DISCUSSION

Three mitochondrial plasmids (mtp1, mtp2 and mtp3) are present in several broad bean lines and have been characterized in detail (5–7). In the line G58 bearing the cytoplasm 350, we have previously identified an additional plasmid (1540 renamed here mtp5), whose restriction map shows it contains a large part of mtp1 (9). In this report, we have shown that cytoplasm 350 contains other additional plasmids, the nature of which varies according to the nuclear background. Eight additional plasmid types (including mtp5 previously cloned) were analyzed in detail. Their complete (mtp 4–6) and partial (mtp 7–11) sequences strongly suggest that they arose by recombination between mtp1 and mtp2. Two successive or simultaneous recombination events must have occurred in order to account for their appearance. One occurred within a 276-bp stretch which is identical in both plasmids. In seven cases out of eight, the other event occurred in a direct repeat region and the recombination point was confined to hexanucleotide GCGACG. The reciprocal molecules which should mainly consist of mtp2 sequences were never found by hybridization. There is no reason to believe that these reciprocal molecules cannot be replicated, so we must consider the possibility of an incomplete exchange leading to only one of two possible molecules. Alternatively, maintenance of the reciprocal molecule could have a detrimental effect on cell development, for instance because the mtp2 transcript level might rise too high.

The second major point reported in this paper concerns the presence or absence of variant plasmids in cytoplasm 350 associated with various nuclei. This was observed in lines resulting from 9–13 backcrosses (Fig. 2). Although there is still a variation of the nature of the additional plasmids among individual plants (Fig. 3), it is clear that additional plasmids were detected with the nucleus 127 or G58 but never with the nucleus 196. This was confirmed in independent crosses. For instance, replacing the G58 nuclear genome with the 196 nuclear genome led to the disappearance of the additional plasmids mtp5 and 6 in all the plants analyzed. The reciprocal cross resulted in new plasmids becoming detectable even if there is a clonal variation. Control of mitochondrial sequences by the nucleus has been demonstrated in several cases (see for instance refs. 15–19). Two explanatory hypotheses are generally proposed: (1) that the nucleus increases the rate of recombination events, for instance by expressing enzymes or factors involved in recombination; (2) that the nucleus controls the level of plasmid molecules. There is now evidence from several sources supporting the existence in plant mitochondria of sublimons, i.e. molecules replicated in sub-stoichiometric amounts (20). Under certain conditions, these low-copy molecules can be amplified and become preponderant. Both hypotheses might apply to our additional broad bean plasmids. Long exposure of Southern blots like those used here failed to reveal the presence of sublimons at the expected size. Such molecules are hard to detect, since plasmids mtp1 and mtp2 present in normal amounts rapidly increase the background of autoradiograms exposed for a long time. However, long exposures did reveal additional hybridization bands in the higher molecular weight range. The low intensity of these bands indicates either that homology is low or concerns a short sequence or that hybridizing sequences are scarce. At this stage we cannot exclude that these faint bands belong to the nuclear genome, since nuclear (but no chloroplastic) sequences do hybridize with mitochondrial plasmids (7). The latter hypothesis seems unlikely, however, since mitochondrial fractions were treated with DNase prior to DNA isolation. The origin of these bands thus requires further investigation.

The observation that the same plasmid (mtp6) is present in two independent backcrosses of 196 with the G58 genome is not proof of the existence of this plasmid as a sublimon. Recombinations could indeed have occurred along a stretch of 276 identical

nucleotides in one case and of 385 nucleotides in the other. The mtp6 molecules of distinct lines might thus have arisen independently, even though their sequence is identical. Among individual plants issued from the fourth backcross, some had detectable amounts of mtp6 while others had very little or none. Again, we cannot tell whether this heterogeneity reflects the variable segregation of a new plasmid created in an ascendant, or different expression levels of a sublimon. However this may be, the existence of several distinct variant plasmids probably reflects an active recombination mechanism. Analysis of 57 wild varieties and cultivars revealed the presence of an mtp2-like plasmid in all of them, thus confirming the prevalence of this plasmid. While an mtp1-like plasmid was found in most varieties, additional plasmids (all the size of mtp6) were found in only three, suggesting that their presence in cytoplasm 350 may be a special case. It is worth recalling that no additional plasmids were ever found in cytoplasm 447, which also confers male sterility and displays a restriction profile identical to that of cytoplasm 350 (11). It would thus be interesting to introduce into cytoplasm 447 the nuclei which allowed detection of variant plasmids in 350. Failure to observe new plasmids after several backcrosses would show that the variant plasmids in 350 are probably sublimons not present in 447.

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