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High-rate spontaneous reversion to cytoplasmic male sterility in sugar beet: a characterization of the mitochondrial genomes

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Summary. Among the fertile sugar beet lines with nuclear sterility maintenance genes, *rf*, in a homozygous recessive state, sublines capable of reverting spontaneously at a high rate to sterility were identified. Of 24 related fertile sublines studied, 6 were found to spontaneously revert to sterility with a frequency of about 19%. Genetic analysis confirmed the cytoplasmic nature of spontaneously arising sterility. Reversion to sterility in these sublines was accompanied by alterations in the mitochondrial genome structure: loss of the autonomously replicating minicircle c (1.3 kb) and changes in the restriction patterns of high-molecular-weight mitochondrial DNA (mtDNA). Southern hybridization analysis with cloned minicircle c as a probe revealed no integration of this DNA molecule into the main mitochondrial and nuclear genomes of the revertants. Comparative BamHI and EcoRI restriction analysis of the mtDNA from the sterile revertants and fertile parental subline showed that the spontaneous reversion is accompanied by extensive genomic rearrangement. Southern blot analysis with cloned α-subunit of F_1-ATPase (*atpA*) and cytochrome c oxidase subunit II (*COX II*) genes as probes indicated that the changes in mtDNA accompanying spontaneous reversion to sterility involved these regions. The mitochondrial genomes of the spontaneous revertants and the sterile analogue were shown to be identical.

Key words: Cytoplasmic male sterility – Mitochondrial genome – *Beta vulgaris* L. – Spontaneous reversion – Minicircle DNA

Introduction

Cytoplasmic male sterility (cms), phenotypically manifested as an inability to produce viable pollen, is the result of the interaction of sterile (S-type) cytoplasm and the recessive nuclear restorer (*rf*) genes. The maternally inherited cms trait has been identified in many plant species (Edwardson 1970; Laser and Lersten 1972). From the recent studies of the mitochondrial and chloroplast genomes of the higher plants, it has been concluded that the genetic determinants of cms reside in the mitochondrial DNA (Hanson and Conde 1985; Lonsdale 1987; Newton 1988).

Comparative studies of the mitochondrial genomes of the sterile and fertile plants have demonstrated rearrangements of their mtDNA and alterations in the transcription and translation patterns. Correlations between cms expression and structural and/or functional alterations in the mitochondrial genome have been reported for maize (Isaac et al. 1985a, b; Abbott and Fauron 1986; Small et al. 1987), sorghum (Dixon and Leaver 1982; Bailey-Serres et al. 1986), sunflower (Siculella and Palmer 1988), bean (Mackenzie et al. 1988), radish (Makaroff and Palmer 1988), and petunia (Young and Hanson 1987).

The sugar beet cms plants from American variety populations identified by Owen (1942, 1945) were the first sources of S-cytoplasm in *Beta vulgaris* L. Subsequently the S-cytoplasm has been identified in other sugar beet populations (Savitsky 1954; Bandlow 1955; Iordansky and Lutkov 1966).

The mitochondrial genome of sugar beet is multipartite consisting of, in addition to high-molecular-weight (HMW) DNA, low-molecular-weight (LMW) minicircle plasmid-like molecules (Powling 1981; Thomas 1986; Mikami et al. 1985; Dudareva et al. 1988a, b; Hallden et al. 1988). The absence of definite minicircles from the mtDNA is characteristic of the sterile sugar beet lines (Powling 1981; Thomas 1986; Dudareva et al. 1988a).

The fertile and cms sugar beet lines differ in restriction endonuclease patterns, showing different distribu-

tion of mitochondrial restriction fragments; the lines also differ in Southern hybridizations of digested mtDNA with cloned mitochondrial genes encoding the subunit II cytochrome c oxidase (*COX II*), apocytochrome b (*COB*), and the α-subunit of F_1-ATPase (*atpA*) (Powling 1982; Powling and Ellis 1983; Mikami et al. 1984; Samoylov et al. 1986; Dudareva et al. 1988 b, 1989).

The data concerning the mtDNA rearrangement in cms plants are abundant. However, the molecular mechanisms underlying the cms trait are unclear. The availability of plants with a high rate of spontaneous reversion to sterility (or to fertility) on unchanged nuclear background may be helpful in understanding the mechanisms of these events. Recently some sugar beet sublines with the needed properties were found. The fertile plants of these sublines (with the *rf* genes in a homozygous recessive state) show a high rate of spontaneous reversion to sterility (up to 19% according to our present estimates).

The aim of the present work was to study whether or not this high rate of spontaneous reversion to sterility is accompanied by the characteristic alterations in the mtDNA structure.

Materials and methods

Plant materials

The male fertile sugar beet lines SOAN-31 homozygous for the *rf* genes were obtained from the collection of the Laboratory of Plant Population Genetics, Institute of Cytology and Genetics, Novosibirsk, USSR. All the sublines were derived from a single plant of variety Uladovskaya odnosemennaya. They were produced by alternation of two forms of inbreeding: selfing (J) and sib-crosses (G). The sublines studied (the J_3G_2 generation, inbreeding coefficient $F=0.916$) had a common pedigree up to the J_2G_2 ($F=0.832$) (Maletsky 1983).

To develop a sterile analogue of SOAN-31, an original cms plant (kindly provided by Prof. R. Melzer, GDR) was backcrossed for six generations to the SOAN-31 line. This sterile analogue was designated as cms-SOAN-31.

Genetic analysis of the spontaneous cytoplasmic revertants was performed with the use of lines of O-type, SOAN-31, -98, and -243 from the collection of the Laboratory of Plant Population Genetics. The plants were hand-pollinated. The branches of the sterile plants were covered with parchment isolators prior to opening of the flowers (20–30 flowers). Several days after flowering, pollen from the open flowers was applied to the stigma with a soft brush. The pollinated flowers were covered with the parchment for the whole period to the maturity of the seeds. The fertile maternals were castrated before involvement in the crosses. The study was performed from 1986 to 1989 at the experimental farm in the region of Prjevalsk. The revertants that arose spontaneously were identified in 1986 among the plants of the fertile SOAN-31 line. In the progeny of this line, a high reversion frequency to sterility was registered continuously throughout the last 6 years.

Identification of sterile plants

The plants were classified for sterility by Owen's microscopic criteria (1945): ms_0, plants with white anthers, very small amounts of pollen, hardly visible on the stigma, without pores, unstainable with acetocarmine; ms_1, plants with pale-yellow anthers, some pollen with well-formed pores, unstainable with acetocarmine. The plants were classified as fertile if any amounts of mature pollen were stained by acetocarmine.

DNA isolation and analysis

MtDNA was isolated from etiolated 5-day-old sugar beet seedlings, as previously described (Dudareva et al. 1988 a, c) and from sugar beet roots, also as described (Rogers and Bendich 1985). Total DNA was isolated according to Rogers and Bendich (1985).

Digestion of mtDNA samples was performed with the BamHI and EcoRI restriction endonucleases under standard conditions. The digested mtDNA samples were electrophoresed in horizontal 0.8% agarose slab gels. Gel electrophoresis of the undigested mtDNA was conducted in 1.5% agarose. DNA in the gels was stained in EtBr, photographed, and transferred to nitrocellulose filter according to Southern (Maniatis et al. 1982). The products of HindIII and EcoRI restriction of phage λ DNA were used as molecular weight standards.

Minicircle c (1.3 kb) from sugar beet mitochondria cloned in pAT153 (a kind gift from Dr. C. Thomas, John Innes Institute, Norwich, UK) and genomic clones of maize cytochrome c oxidase subunit II (*COX II* pZME1) and the α-subunit of F_1-ATPase (*atpA*-copy V) (kindly provided by Dr. C.J. Leaver, Edinburgh University, Scotland) were used as probes in the Southern hybridizations.

Integrated minicircle c and mitochondrial gene sequences were recovered from the recombinant plasmids and labeled by random priming, using [α-^{32}P]-dATP (the specific activity of the probe was $1-5\times10^8$ cpm per μg DNA). The probes were applied in individual blot hybridizations.

The hybridization medium contained: 2× Denhardt's solution (50× stock = 1% w/v Ficoll, 1% w/v PVP and 1% BSA), 5× SSPE (20× stock = 3.6 *M* NaCl, 0.1 *M* sodium phosphate, pH 7.4, 0.02 *M* EDTA), 50% formamide, 100 μg/ml denatured sonicated salmon sperm DNA, and radioactive probe.

After hybridization, the filters were washed in 2× SSC (20× stock = 3 *M* NaCl, 0.3 *M* sodium citrate, pH 7.0), 0.1% SDS for 20 min. The washings were done three times each at room temperature and at 65 °C. The filters were autoradiographed in X-ray film at −70 °C.

Results

Genetic analysis of sterile revertants

The sterile revertants, which arose spontaneously in the fertile SOAN sugar beet lines, were studied. High reversion frequency to sterile phenotype was determined in 6 of 24 fertile sublines. In these 6 lines, the percentage of sterile plants was 19%, on the average (Table 1). The phenotype of the sterile plants was easily identified by pollen production according to Owen's classification.

The genetic analysis of the sterile revertants was carried out with the use of three O-type test lines: SOAN-31, -98 and -243 (Collection of the Laboratory of Plant Population Genetics). No case of spontaneous occurrence of sterile revertants in these three lines was observed. SOAN-243 did not restore to full sterility (Table 2).

Table 1. Distribution of sugar beet plants with cytoplasmic male sterility among fertile lines SOAN-31

Sublines	No. of plants	
	Sterile	Fertile
SOAN-31-17	7	23
SOAN-31-19	3	2
SOAN-31-25	1	8
SOAN-31-39	5	51
SOAN-31-44	6	9
SOAN-31-60	3	13
Total	25	106
Frequency	19%	81%

Table 2. Phenotypic classification of the F_1 hybrid based on pollen sterility

Maternal plants	Pollinators (sterility maintainers)					
	SOAN-31[a]		SOAN-98		SOAN-243	
	Phenotype F_1 (no. of plants)					
	ms	f	ms	f	ms	f
ms_{rev} SOAN-31-19	26	–	25	–	24	–
ms_{rev} SOAN-31-25	5	–			17	11
ms_{rev} SOAN-31-39	15	3	10	–	6	10
ms_{rev} SOAN-31-60	9	–	26	–	11	–
cms-SOAN-31-25	22	–	40	–	32	4
SOAN-31-25					–	17

ms: male-sterile plants; f: male-fertile plants
[a] ms_{rev} SOAN-31-19 was pollinated with SOAN-31-33
ms_{rev} SOAN-31-25 was pollinated with SOAN-31-25
ms_{rev} SOAN-31-39 was pollinated with SOAN-31-39
ms_{rev} SOAN-31-60 was pollinated with SOAN-31-60

Two ms plants with revertant cytoplasm (ms_{rev}SOAN-31-19 and ms_{rev}SOAN-31-25) were phenotypically classed as ms_0 according to Owen (1945). The hybrid progeny from crosses of the ms_{rev} SOAN-31-19 with each of the three lines was sterile; of the 75 sterile hybrids, only 2 (the cross ms_{rev} SOAN-31-19 × SOAN-98) were classed as ms_1. The hybrid progeny from the cross ms_{rev} SOAN-31-25 × SOAN-31-25 (the sister plant cross) gave 5 sterile plants (2 ms_0 and 3 ms_1 classes), and that from the cross ms_{rev} SOAN-31-25 × SOAN-243 yielded only 17 sterile and 11 fertile plants; the pollen was 5%–100% fertile in these fertile plants. In contrast, the progeny from the control crosses of two fertile lines (the cross SOAN-31-25 × SOAN-243) was all fertile. Thus, we had assurance that the sterile plants with revertant cytoplasm are cms because their sterility is maternally inherited.

Variation in plasmid-like minicircle composition in the mtDNA of fertile sugar beet line and sterile cytoplasmic revertants

Gel electrophoresis of total mtDNA from higher plants in 1.5% agarose makes it possible to separate the bulk of the high-molecular-weight mtDNA (at the top of the gel) from a set of minicircle low-molecular-weight DNA molecules (at the bottom of the gel). Fig. 1 A shows an electrophoregram of the mtDNA from plants of fertile line SOAN-31-25 and the spontaneous revertant ms_{rev} SOAN-31-25. The mitochondria of the fertile plants (N-cytoplasm) contain two types of low-molecular-weight DNA molecules, and those of the sterile plant one only. The same was observed for the mtDNA from the other two revertants, ms_{rev} SOAN-31-39 and ms_{rev} SOAN-31-60. These low-molecular-weight DNAs in the fertile sugar beet plants are double-stranded, supercoiled circle molecules designated as a and c (mc a and mc c), 1.6 kb and 1.3 kb in size, according to the observations of Powling (1981), Hallden et al. (1988, 1989), and Dudareva et al. (1988 a, c).

There are comparative electrophoretic data indicating variations in the set of plasmid-like minicircle DNA in the mitochondrial genome of the fertile and sterile sugar beet plants: the minicircle DNA molecules of 1.3 and 1.4 kb, consistently present in the fertile lines, are absent from all the studied cms lines that retain only 1.6-kb DNA molecules (Powling 1981; Dudareva et al. 1988 a, b). The present data demonstrate that the sterile revertants have only the 1.6-kb minicircle DNA molecule, exactly like cms plants.

There may possibly be two reasons why mc c is absent from the mtDNA from the sterile revertant. The mc c concentration may be so low as to elude detection in the

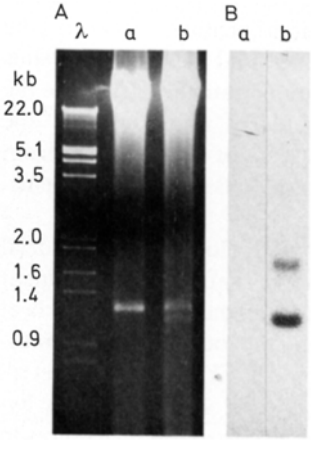

Fig. 1 A and B. Analysis of total undigested mtDNA from spontaneous revertant ms_{rev} SOAN-31-25 and its fertile parent SOAN-31-25 in 1.5% agarose gel (**A**) and hybridization with cloned mc c (**B**). *Lane a* – mtDNA from ms_{rev} SOAN-31-25; *lane b* – mtDNA from fertile line SOAN-31-25

1.5% agarose slab gels, or mc c might have integrated into the main mitochondrial or nuclear genomes.

To verify these suggestions, total undigested mtDNA from fertile line SOAN-31-25 and its sterile revertant were loaded onto 1.5% agarose gels, transferred to nitrocellulose filters, and then Southern blotted, using mc c as a hybridization probe (Fig. 1 B). No hybridization of mc c to the Southern blots of the mtDNA from the spontaneous sterile revertant ms_{rev} SOAN-31-25 occurred, confirming the absence of sequences homologous to mc c from the main mitochondrial genome.

We then sought to determine whether or not the mc c had integrated into the nuclear genome. After separation on 0.8% agarose gel of EcoRI digest of total DNA from the fertile SOAN-31-25 plant and its sterile revertant, a Southern blot containing the digest was hybridized to mc c. Here again, no hybridization of mc c to the Southern blots of total DNA from the fertile line and its revertant occurred (data not shown). These data clearly demonstrate that mc c had not integrated into the main mitochondrial or nuclear genomes.

Analysis of mtDNA rearrangement associated with spontaneous reversion to sterile phenotype

The next question was whether or not spontaneous reversion to cytoplasmic male sterility in the sugar beet plants was associated with rearrangements in the main mitochondrial genome. To answer this question, the following experiments were performed. The mtDNA was isolated from the fertile line SOAN-31-25, its sterile revertant ms_{rev} SOAN-31-25, and also from the fertile SOAN-31-60 and its sterile revertant ms_{rev} SOAN-31-60. Isolated mtDNAs from the lines were compared by restriction analysis, using restriction endonucleases BamHI and EcoRI, and the fragments of the digest were electrophoresed in 0.8% agarose gel. There were numerous differences in the restriction profiles between the mtDNAs from the fertile lines and their spontaneous revertants. Fig. 2 A and B demonstrate the pattern on the restriction fragments. Particularly noteworthy is the identity of the restriction patterns of the mtDNAs from the spontaneous sterile revertant and the sterile line cms-SOAN-31.

Using blot hybridization with cloned mitochondrial genes, we have previously shown that the rearrangement of the mitochondrial genomes of the sterile sugar beet lines has the effect of changing the locations of the mitochondrial genes *COB*, *atpA*, and *COX II* (Dudareva et al. 1988 b). The use of the genes *COX II* and *atpA* as hybridization probes allowed us also to detect differences in the primary structure of the mtDNA from the sterile lines of different origin (Dudareva et al. 1989). We used, as the most informative, mitochondrial genomic clones carrying the coding sequences for the subunit II of cytochrome

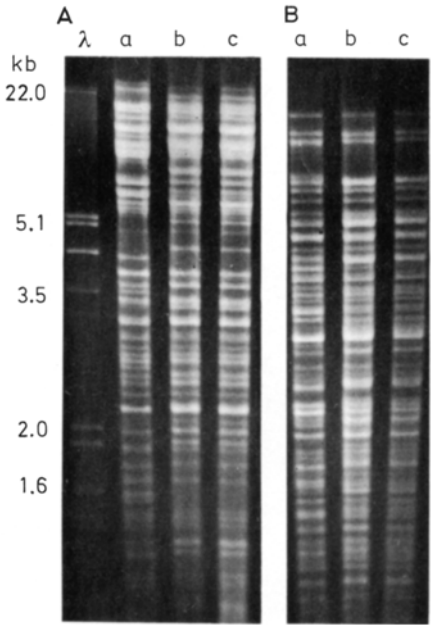

Fig. 2 A and B. Restriction analysis of mtDNA from fertile line SOAN-31-25 (*lane a*), its sterile revertant ms_{rev} SOAN-31-25 (*lane b*), and sterile line SOAN-31 (*lane c*). DNAs were digested with **A** BamHI and **B** EcoRI and electrophoresed on a 0.8% agarose gel

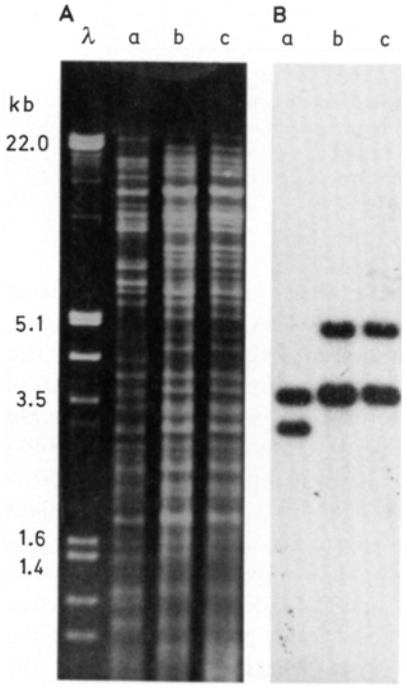

Fig. 3 A and B. Southern blot (**B**) of mtDNA digested with BamHI (**A**) from fertile line SOAN-31-25 (*lane a*), sterile analogue cms-SOAN-31 (*lane b*), and spontaneous sterile revertant ms_{rev} SOAN-31-25 (*lane c*) probed with radiolabeled *atpA* gene

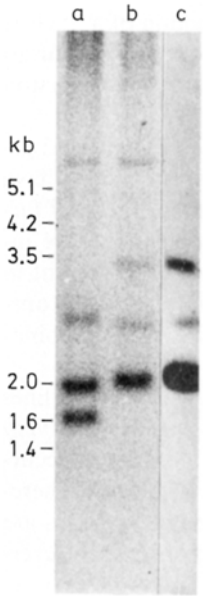

Fig. 4. Southern blot of mtDNA digested with BamHI from fertile line SOAN-31-25 (*lane a*), spontaneous sterile revertant ms_{rev} SOAN-31-25 (*lane b*), and sterile analogue cms-SOAN-31 (*lane c*) probed with radiolabeled *COX II* gene

c oxidase, and the α-subunit of F_1-ATPase for blot hybridization analysis of the genomic rearrangement in the spontaneous sterile revertant ms_{rev} SOAN-31-25, in comparison to the fertile SOAN-31-25 line and its sterile counterpart, cms-SOAN-31. The results of blot hybridization of total mtDNA BamHI digest probed with the *atpA* cloned fragment are seen in Fig. 3 B. The BamHI digest shows a major band of 3.5 kb hybridizing with the *atpA* probe. This band is present in the profiles of all the mtDNA digests from the fertile and sterile plants. A band of about 3.0 kb is observed only in mtDNA from the fertile plants, and a band of about 5.0 kb is present in the mtDNA only from the sterile plants. The *atpA* gene of the sterile revertant mtDNA and of the sterile line cms-SOAN-31 is located in the same region of the main mitochondrial genome (Fig. 3 B). The hybridization profiles are identical with those obtained earlier for the fertile and sterile *Beta vulgaris* L. lines of different origin (Dudareva et al. 1989).

We further examined the differences in the mtDNA sequences from the sterile revertant and its fertile parent using *COX II* as a probe (Fig. 4). As Fig. 4 shows, the sterile revertant differs from the fertile parent (lane b) in the absence of a parental-specific 1.6-kb band and the presence of a new 3.5-kb band. Similar data were obtained earlier for the location of the *COX II* gene in the mtDNA from sterile sugar beet plants (Dudareva et al. 1989). Thus, the reversion to sterility in the studied sugar beet lines is accompanied by a reorganization of mtDNA sequences, and the mitochondrial genomes of the revertants become identical with that of the standard sterile plants.

Discussion

The phenomenon of the spontaneous reversion to sterility or, conversely, to fertility offers promise as a genetic model in investigating the nature of cytoplasmic male sterility and nucleo-cytoplasmic interaction.

In the present study of the fertile SOAN-31 sugar beet lines, we identified lines able to revert spontaneously to sterility at a high rate. Of the 24 related fertile sublines, 6 were found to spontaneously revert to sterility at a frequency of about 19%; the remaining 18 showed no reversion. The results of the genetic analysis of the progeny from the self-pollination of the sterility restorers SOAN-31-19 and SOAN-31-25 (plants with N-cytoplasm) follow. In the progeny, along with fertile plants, sterile plants appeared as a result of cytoplasmic reversion to sterility (N→S).

Comparative electrophoretic analysis of the mtDNA from the parental fertile line and its spontaneous revertant demonstrated variations in the set of plasmid-like minicircle DNA molecules: mc c was absent from the mtDNA of the revertants. We did not find integration of the mc c into the main mitochondrial genome as in the case of plasmid-like DNAs S1 and S2 in maize fertile cytoplasmic revertants (Kemble and Mans 1983), nor did we find integration of mc c into the nuclear genome. The mechanism of the elimination of mc c as a result of reversion to sterility is unknown. The possibility cannot be excluded that a disturbance of its autonomous replication might have resulted in elimination of mc c in the cms revertants.

The array of linear and circular DNA plasmids found in the mitochondria of higher plants is variable (Pring and Lonsdale 1985; Newton 1988). There exist, in addition to HMW molecules, LMW molecules, and their replication is thought to be autonomous of the HMW genome. The plant species in which the presence or absence of the plasmid-like molecules and the expression of the cms are associated include maize (Pring et al. 1977), sorghum (Pring et al. 1982; Dixon and Leaver 1982), bean (Boutry and Briquet 1982; Goblet et al. 1985), and rice (Mignouna et al. 1987; Nawa et al. 1987). However, this association has not been clarified in cause-effect terms.

In the sugar beet mitochondrial genome, four types of minicircle DNA were detected: mc a, 1.6 kb; mc b, 1.45 kb; mc c, 1.4 kb; and mc d, 1.3 kb (Powling 1981). Although no homology between the minicircles and the main mitochondrial genome is observed, minicircles show homology with each other (Hansen and Marcker 1984; Thomas 1986). Knowledge about the origin of these molecules, their function, and the relation to the

expression of cms is limited. However, correlation between the presence of minicircle DNA of a certain size and cms expression has been repeatedly observed (Powling 1981; Hansen and Marcker 1984; Thomas 1986; Dudareva et al. 1988a, b). Thus, there was a concomitance in the presence of mc a and absence of mc b, c, and d in the sterile sugar beet lines, the three latter being consistently present in different combinations in the fertile sugar beet lines.

The present results indicate that the spontaneous reversion of the normal to sterile cytoplasm (N→S) in sterility restorer plants is accompanied by disappearance of mc c. There are no good reasons for regarding this disappearance as requisite for the spontaneous N→S reversion or, moreover, as causative.

Comparative restriction analysis of the mtDNA from the sterile revertant and fertile parental lines demonstrated that spontaneous N→S reversion is accompanied by an extensive genomic alteration. This genomic alteration resulted in change of the location of the *COX II* and *atpA* genes in the sterile revertant, as revealed by Southern analysis. The present results are in line with the growing body of evidence indicating that each and every cms mtDNA displays alterations in the restriction profile relative to the normal, and that these alterations are due to rearrangements and, most importantly, that they are features common to all cms plants. It is pertinent to recall that mitochondrial gene alterations associated with cms were reported for maize (Isaac et al. 1985a, b; Small et al. 1987), sorghum (Bailey-Serres et al. 1986), sunflower (Siculella and Palmer 1988), radish (Makaroff and Palmer 1988), and petunia (Young and Hanson 1987). Rearrangements in the cms-T cytoplasm of maize gave rise to a novel gene comprising DNA fragments of the flanking or coding regions of the 26 *S* rRNA, *atp6*, and chloroplast $tRNA^{Arg}$ genes (Dewey et al. 1986). This new recombinant gene encodes a 13-K protein whose presence is associated with cms expression and toxin susceptibility (Dewey et al. 1987, 1988). A mitochondrial genome rearrangement in sorghum presumably associated with cms leads to altered transcription and translation of the *COX I* gene and, as a consequence, a 42-K modified protein corresponding to *COX I* is synthesized instead of the normal 39-K protein (Dixon and Leaver 1982; Bailey-Serres et al. 1986). The synthesis of the modified protein is produced by a mutation in the coding region of the *COX I* gene. The chimaeric gene *Pcf* containing the 5′-flanking region of *atp9*, portions of the *COX II* coding region, and the 3′-flanking region of an unidentified reading frame is transcribed only in the cms lines of petunia (Young and Hanson 1987).

Reorganization of mtDNA associated with cytoplasmic reversion to fertility has been recently examined in maize (Escote-Carlson et al. 1988) and *Phaseolus vulgaris* (Mackenzie et al. 1988). The mitochondrial genomic rearrangements produced by the introduction of a nuclear restorer gene into the cms line genome are similar to those occurring upon spontaneous cytoplasmic reversion to fertility.

The alterations we observed in the mitochondrial genomic organization of the sterile revertant might have been the result of recombinations in the mtDNA of fertile normal plants. These rearrangements may have included deletions or insertions; direct repeats, present in the sugar beet mitochondrial genome (Brears and Lonsdale 1988), may have been involved in the recombinational events.

It is noteworthy that only 6 of the 24 fertile lines examined reverted spontaneously to sterility at a very high frequency, surpassing by several orders the occurrence probability of natural mutational events. Therefore, it may be assumed that recombination systems are highly active in the mitochondria of spontaneous revertants.

An important feature of the sugar beet mtDNA is that it contains five direct repeats varying from 0.5 to 6 kb. It thus seems probable that the mitochondrial genome has large amounts of enzymes providing a high level of homologous recombinations predetermined by the relative position of the direct repeats. On the other hand, we cannot exclude the possibility that some mutations occurring in the nuclear genome are favorable for the maintenance of mtDNA of sterile type.

As a result of selection, other possible recombinations were eliminated from the revertant, and there remained precisely the mtDNA rearrangement leading to the cms state without affecting other traits and vital systems. The important feature of the spontaneous reversion of sterility is a reorganization of the mitochondrial genome in the sterile revertant that is identical with the one occurring in its sterile counterpart.

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