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Expression of mitochondrial genes in fertile and sterile sugar beet cytoplasms with different nuclear fertility restorer genes

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Summary. Variations in mitochondrial genome organization and in its expression between fertile, sterile sugar beet lines and fertile nuclear-restored plants were studied. Southern blot hybridization with *COXI, COXII, COB* and *atpA* mitochondrial genes as probes showed that changes in the mitochondrial genome organization of sterile lines are associated with variations in the location of *COB, atpA* and *COXII,* but not *COXI.* When the *COXII* and *atpA* genes were used as hybridization probes, differences in the primary structure of mitochondrial DNAs from sterile lines of different origin were revealed. Differences in the transcriptional patterns of the three mitochondrial genes (*COXI, COXII* and *atpA*) were observed between fertile and sterile sugar beet lines; *COB* was the only mitochondrial gene whose transcription was identical in both fertile and sterile cytoplasms. The dominant nuclear fertility restorer genes altered the transcriptional patterns of the *COB* and *atpA* without affecting those of the *COXI* and *COXII* genes; *atpA* expression was identical in fertile plants and nuclear-restored plants with sterile cytoplasm.

Key words: Cytoplasmic male sterility – *Beta vulgaris* L. – Mitochondrial DNA – Mitochondrial gene expression, nuclear restorer genes

Introduction

Cytoplasmically inherited male sterility (cms) is a widely occurring phenotype in higher plants that is characterized by a failure to produce functional pollen (Edwardson 1970; Laser and Lersten 1972; Kaul 1988). This trait enables the plant breeder to produce F_1 hybrid seed varieties without time-consuming castration and under conditions of controlled pollination.

Nuclear pollen sterility is caused by mutations of the nuclear genes, cms, in contrast, is the result of the interaction of specific recessive nuclear genes, the nuclear restorer of sterility (*rfrf*) genes, and a specific, different from normal (N), sterile (S) cytoplasm. The effect of the *rf* genes is not expressed in N type cytoplasm: in fact, plants with the normal type of cytoplasm are consistently fertile. However, the dominant nuclear genes (*RfRf*) interact specifically with the sterile cytoplasm and thereby suppress its expression. Crossing of fertile lines having the dominant nuclear restorer of fertility genes with sterile lines having S-cytoplasm and *rfrf* nuclear genes yields an F_1 hybrid progeny of fertile plants with sterile cytoplasm (S-cytoplasm and *Rfrf* nuclear genes).

The maternal inheritance of cms implies that, in principle, any genetic element of the cytoplasm, the mitochondrial or chloroplast genome, can carry the determinants of the cms trait. In recent years, a convincing body of evidence has been accumulated that strongly suggests that the genetic determinants of the cms trait in higher plants resides on the mitochondrial DNA (mtDNA) (Hanson and Conde 1985; Lonsdale 1987; Newton 1988; Leaver et al. 1988; Levings and Brown 1989).

While the identification of the first sterile plants in a sugar beet population dates back to 1942 (Owen 1942), we still know little about the molecular mechanisms of the cms trait in sugar beet and other plants. The structure of mitochondrial genomes in plants has been intensively studied in recent years. The size of the sugar beet mitochondrial genome has been estimated to be 386 kb, and its structure and organization is more complex than those of the dicotyledonous plants studied so far. The mtDNA consists of a master chromosome, which con-

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tains five repeats of 6, 4, 1, 0.5 and 0.5 kb, and also a population of subgenomic molecules that have arisen as a result of recombination events between the repeats (Brears and Lonsdale 1988).

Restriction and hybridization analyses consistently reveal significant structural differences in the master mitochondrial genomes between sterile and fertile lines of sugar beet (Powling 1982; Powling and Ellis 1983; Mikami et al. 1984; Samoylov et al. 1986; Duchenne et al. 1989; Dudareva et al. 1989, 1990; Komarnitsky et al. 1990).

We present here the results of our analysis of the structure of mtDNA and transcriptional patterns of the mitochondrial genes encoding the subunits I and II of the cytochrome c oxidase complex (*COXI* and *COXII*), apocytochrome b of the bc1 complex (*COB*) and the α-subunit of the F_1-ATPase (*atpA*) in fertile and sterile sugar beet isolines. The effect of nuclear fertility restorer genes on the expression of the mitochondrial genes studied was also examined.

Materials and methods

Plant materials

The following sugar beet (*Beta vulgaris*) lines were used in this study: sterility maintainers SOAN-31, -99, -103, -234, – 46A-15; sterile line cms-SOAN-31 (collection of the Laboratory of Plant Population Genetics, Institute of Cytology and Genetics, Novosibirsk, USSR), sterile forms 83138 (Uladovskaya Experimental Breeding Station) and 8576 (All-Union Sugar Beet and Sugar Research Institute).

Fertile line SOAN-31 of the O-type was derived from the variety 'Uladovskaya odnosemennaya' by alternating two forms of inbreeding, selfing (the J generation) and sib-crossing (the G generation). The subline used in this study was of the J_3G_3 generation (inbreeding coefficient $F=0.932$) (Maletsky 1983).

A sterile analogue of line SOAN-31 was developed from an original cms plant kindly supplied by Prof. R. Melzer (FRG). The cms plant was backcrossed for six generations to SOAN-31, and the resultant plants will be henceforth designated as cms-SOAN-31.

To derive fertile plants with S cytoplasm from cms-SOAN-31, we utilized line SOAN-76 (the J_2G_2 generation, $F=0.832$) as the fertility restorer. SOAN-76 was developed from variety 'Ramonskaya 09'. The inbred J_2 population was developed through selfing fertile plants of the J_0 and J_1 generations.

The roots of each of six J_2 plants were cut into two halves: one-half was used to study the transcriptional activity of mtRNA; the other half served as control, i.e. it was planted in the greenhouse, and the pollen of the grown plants was evaluated for fertility.

The J_0 generation was obtained by hand-pollinating of plants. The sterile plantings (20–30 flowers) were bagged with parchment to prevent pollination from stray pollen. Pollen was collected a few days after the opening of the flowers and distributed over the stigmas with a soft brush. Then the flowers were rebagged. The selfing of plants was performed under cloth bags. All the hybridizations were performed in the fields located at high altitudes (Prjevalsk, Kirghizstan).

Mitochondrial gene probes and ^{32}P-labeling

The following maize mitochondrial gene cloned into plasmids were used as hybridization probes in the experiments: subunit I (*COXI* pBN 6601) (Isaac et al. 1985b) and subunit II (*COXII* pZME 1) (Fox and Leaver 1981) of the cytochrome c oxidase complex, apocytochrome b (*COB* pZMEM 680) (Dawson et al. 1984), and the F_1-ATPase α-subunit (*atpA* copy V) (Isaac et al. 1985a), a kind gift of Dr. C. J. Leaver (Edinburgh University, Scotland).

To obtain ^{32}P-labeled probes, plasmids containing the cloned genes were digested with appropriate restriction endonucleases; after electrophoresis the mitochondrial genes were recovered by electroelution from agarose gels (Maniatis et al. 1982) and labeled by random priming (Feinberg and Vogelstein 1983).

MtDNA extraction and Southern hybridization

Mitochondrial DNAs were isolated from 5-day-old etiolated sugar beet seedlings (Dudareva et al. 1988) or roots as described elsewhere (Weihe et al. 1991). Restriction endonuclease digestion and electrophoresis were performed as described previously (Dudareva et al. 1990). DNA restricts from the gels were transferred to nylon filters (Hiu Kalur, USSR) with the use of Vacu Gene (LKB, Sweden) in 1.5 *M* NaCl, 0.5 *M* NaOH followed by neutralization of the membrane filter in 1 *M* NH_4Ac, 40 m*M* NaOH.

Southern blot hybridization was carried out as previously described (Dudareva et al. 1990).

MtRNA extraction and Northern hybridization

The mitochondrial fraction for mtRNA extraction was obtained from sugar beet roots in the same way as for mtDNA. The purified mitochondria were lysed in a buffer containing 2.5% sodium sarkosylate, 25 m*M* TRIS-HCl, pH 7.5, and 20 m*M* EDTA. Lysis was followed by deproteinization with a phenol: chloroform mixture (1:1). The mtDNA contaminants were removed from the RNA preparations by extraction with an equal volume of phenol saturated with 0.3 *M* NaAc, pH 5.2. After precipitation with ethanol, mtRNA was further purified by resuspending the precipitate 2 times in 3 *M* NaAc, pH 5.2.

Denaturation of mtRNA and electrophoresis in the denaturating 1.2% agarose formamide gel were performed according to Lehrach et al. (1977). The gels were transferred to nylon filters (Hiu Kalur, USSR) in 10 × SSC with the use of Vacu Gene (LKB, Sweden). Before the transfer the gels were washed in 0.1 *M* NH_4Ac to remove excess of formaldehyde.

Northern prehybridization and hybridization were performed at 40 °C in 50% formamide, 0.5% SDS, 5 × Denhardt's solution (50 × stock = 1% w/v Ficoll, 1% w/v PVP and 1% BSA), 5 × SSPE (20 × stock = 3.6 *M* NaCl, 0.2 *M* sodium phosphate, pH 7.4, 0.02 *M* EDTA) and 100 μg/ml denaturated sonicated salmon sperm DNA.

The filters were washed after hybridization by the same procedure as for Southern hybridization (Dudareva et al. 1990).

Results

Comparative hybridization analysis of mtDNA from fertile and male-sterile sugar beet

To study structural changes in the sugar beet mitochondrial genome, which were possibly related to the cms trait, we attempted to determine the location of four mitochondrial genes encoding subunits I (*COXI*) and II

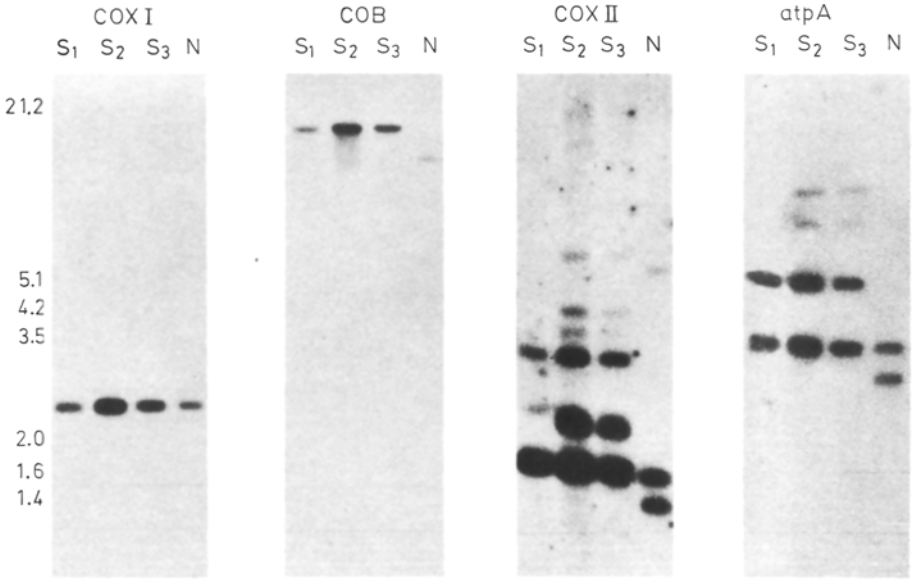

Fig. 1. Hybridization patterns of COXI, COB, COXII and atpA probes with BamHI-digested mtDNA from sugar beet lines. S_1 sterile cms-SOAN-31, S_2 sterile 83138, S_3 sterile 8576, *N* fertile SOAN-31

(*COXII*) of cytochromoxidase c, apocytochrome b (*COB*) and the α-subunit of the F_1-ATPase complex (*atpA*) on mtDNA restricts from three independently derived sterile and five fertile sugar beet lines. We chose lines of different origin in order to identify possible polymorphism in the fertile and sterile cytoplasms. All the fertile lines used were O-types, i.e. sterility maintainers (*rfrf*). We did not observe any polymorphism among the examined fertile sugar beet lines; Fig. 1 shows the results of the hybridization analysis of mtDNA for a single fertile line, SOAN-31. Different ^{32}P-labeled gene-specific probes were hybridized with Southern filters containing the BamHI restricts of mtDNA from fertile and sterile sugar beet lines.

The BamHI probe revealed no differences in the location of the *COXI* gene amongst the mtDNAs from fertile and sterile lines (Fig. 1); the same result was also observed for the SalI and HindIII fragment location of *COXI* (data not shown). As to the BamHI fragment location of *COB*, the fertile lines differed from the sterile lines: a fragment of about 15 kbp hybridized with *COB* in the mtDNA of the fertile lines; a different fragment of about 20 kbp hybridized with *COB* in the mtDNA of the three different sterile lines. As Fig. 1 shows, there were no differences in BamHI fragment location between the sterile lines of different origin.

When the ^{32}P-labeled *COXII* and *atpA* genes were used as hybridization probes, differences in the primary structure of mtDNA were observed not only between the fertile and sterile lines, but also between the sterile lines of different origin. The *atpA* probe hybridized strongly with two BamHI fragments (3.5 and 3.0 kbp) from mtDNA of the fertile lines and also with two fragments (5.0 and 3.5 kbp) in the mitochondrial genomes of the sterile lines (Fig. 1). These results demonstrate that there is a fragment common to all of the fertile and sterile lines tested and that this fragment hybridized with the *atpA* probe. The sterile lines differed in the location of the *atpA* gene: two of the three studied lines (S_2 and S_3) contained two mtDNA fragments of the same high molecular weight. These fragments hybridized to a lesser extent with the *atpA* gene than the other two fragments (5.0 and 3.5 kbp).

The *COXII* probe hybridized with the two BamHI fragments only in the analyzed fertile lines (the mtDNA fragments were of 2.0 and 1.6 kbp) (Fig. 1). The mitochondrial genomes of the sterile lines of different origin differed significantly in their hybridization with the *COXII* probe: two BamHI fragments of mtDNA from sterile cytoplasm S_1 and three BamHI mtDNA fragments from the cytoplasms of two other sterile lines (S_2 and S_3) strongly hybridized with the *COXII* probe.

Thus, blot hybridization with the mitochondrial genes encoding the electron transport chain proteins demonstrated differences in the structure of the mitochondrial genomes of the fertile and different sterile lines in hybridizations with the *COB*, *atpA* and *COXII* genes.

Transcription of the mitochondrial genes in fertile and sterile sugar beet lines

To elucidate the transcriptional patterns of the protein-coding mitochondrial genes, we carried out Northern blot analyses of mtRNA from fertile and sterile sugar beet lines. The Northern blots were hybridized with four cloned mitochondrial genes *COXI, COXII, COB* and *atpA*.

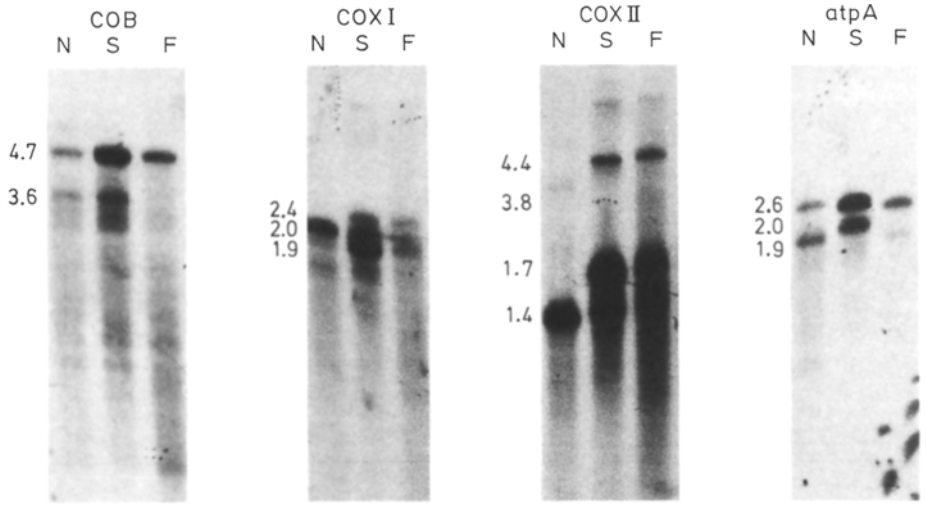

Fig. 2. Mitochondrial transcriptional patterns of the COB, COXI, COXII, and atpA genes in normal fertile (*N*) SOAN-31, sterile (*S*) cms-SOAN-31 and nuclar-restored (*F*) sugar beet lines

Similar transcriptional patterns were observed only for hybridization with the *COB* probe (Fig. 2, lanes N and S): only two transcripts of 4700 and 3600 nucleotides were found to be present in the fertile and sterile cytoplasms. Differences in transcriptional patterns were observed for the other three mitochondrial genes *COXI, COXII,* and *atpA*. A highly abundant *COXI* transcript (about 2000 nt) is specific to normal fertile cytoplasm of sugar beet, two additional highly abundant *COXI* transcripts (2400 and 1900 nt) were identified in sterile cytoplasm (Fig. 2). It should be noted that fertile cytoplasm also contains a transcript of 1900 nt, but it is less abundant than its counterpart in sterile cytoplasm.

Hybridization of the *COXII* probe onto a Northern blot of mtRNAs from fertile and sterile sugar beet lines demonstrated that the transcription of the *COXII* gene is different in N and S cytoplasms. Fertile (N) and sterile (S) sugar beet lines sharply differed in their number of synthesized *COXII* transcripts. Three highly abundant transcripts of 4400, 1700, and 1400 nt were synthesized in sterile cytoplasm, whereas a single transcript of 1400 nt was present in the cytoplasm of the normal fertile line (N). Differences in this respect were also observed for the less abundant *COXII* transcripts.

As to the transcription of the *atpA* gene, two transcripts regularly occur in the fertile and sterile cytoplasm: one of 1900 nt in fertile cytoplasm, one of 2000 nt in sterile cytoplasm and one of 2600 nt common to both cytoplasms.

Effect of the nuclear fertility restorer genes on the expression of the mitochondrial genes

In our experiments we used six sugar beet plants with sterile cytoplasm and restored fertility (Table 1). Line SOAN-76 is not a full restorer of fertility. It should be noted that incomplete fertility restoration is an event common to inbred sugar beet populations (Maletsky et al. 1988). The family we chose had a progenitor whose J_0 generation had a plant with a low percentage of pollen fertility, although in some of the other plants of this generation pollen fertility was complete. This allowed us to expect that the restorer effect may be mainly caused by the restorer genes and not by an alleged set of modifier genes. In generation J_1 we took a plant with 70% viable pollen for self-pollination. In six plants of the J_2 generation, the percentage of fertile pollen would then be 60–100%. These plants would be both homo- and heterozygous for the *Rf* genes, the genes for fertility restoration of pollen. These plants will be henceforth designated as *SRf-*.

Table 1. Derivation of fertile sugar beet plants with male-sterile cytoplasm from the cms-SOAN-31 line

Generation	Number of fertile plants[a]	mcI[b]	mc0[c]
J_0 cms-SOAN-31 × SOAN-76	43	10	2
J_1	36	11	10
J_2	6		

[a] Plants with yellow anthers and pollen grains that stain with any amount of acetocarmine
[b] Plants with yellowish-brown anthers and pollen grains with well developed micropores that do not yet stain with acetocarmine
[c] Plants with pale anthers with either small amounts of pollen grains or empty poreless pollen grains not staining with acetocarmine

To assure ourselves that the cytoplasm of the examined fertile (nucleus-restored) plants was indeed sterile, we analysed the mtDNA from a portion of the roots of these plants. We compared the BamHI digest of mtDNA from all six fertile plants. Figure 3A shows, firstly, that there are no apparent differences in the restriction pro-

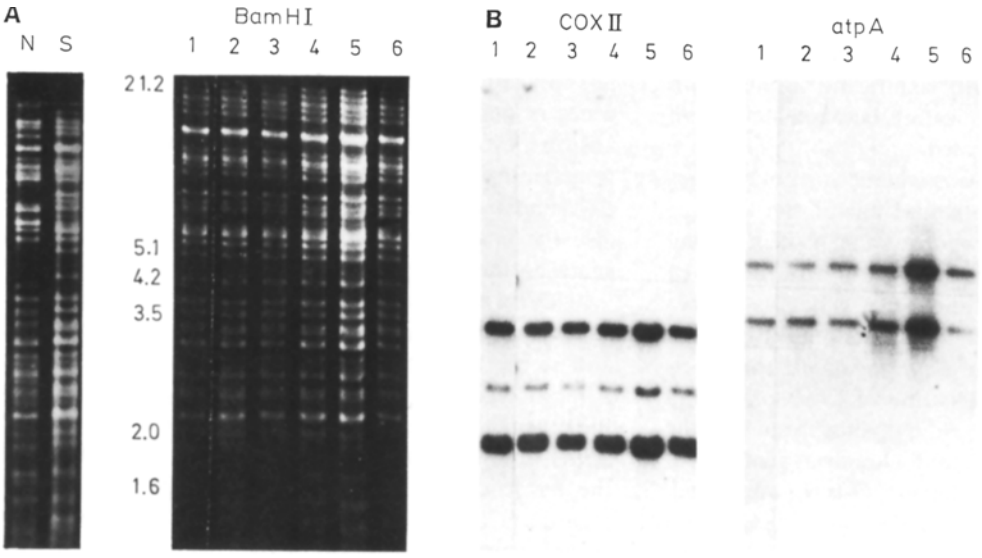

Fig. 3. Restriction (**A**) and hybridization (**B**) analyses of six nuclear-restored sugar beet plants with sterile cytoplasm

files of the six mtDNAs and, secondly, that the restriction profiles obtained are identical to the ones observed for the sterile cytoplasm.

Information helpful in the identification of fertile and sterile cytoplasms was provided by hybridization analyses of mtDNAs with the *COXII* and *atpA* probes (see Fig. 1). We therefore attempted to determine the location of the *COXII* and *atpA* genes in the mitochondrial genomes of the six fertile (nuclear-restored) plants.

The Southern blots showed that the *COXII* and *atpA* genes in mtDNAs from the fertile plants are located at a position specific to sterile (S_1) cytoplasm (Figs. 1, 3 B). Taken together, the results of restriction and hybridization analyses indicated that the fertile (nuclear-restored) sugar beet plants contained sterile cytoplasm.

We next raised the question of whether or not the nuclear genome may affect the expression of the mitochondrial genes. Answers were obtained as follows. MtRNA was isolated from a mixture of six roots of nuclear-restored plants. The results of Northern hybridization with the use of the mitochondrial genes as probes are demonstrated in Fig. 2. Clearly, the nuclear genome has no effect on the transcripts of the *COXI* and *COXII* genes: the transcriptional patterns for plants with restored fertility are identical to those for the sterile plants.

However, it was found that the nuclear genome affects the expression of the other two mitochondrial genes, *COB* and *atpA*. In the case of *COB*, the smaller transcript of 3600 nt, which is present in both sterile and fertile cytoplasms, is missing; as for *atpA*, the transcriptional pattern is identical to the one observed for the fertile phenotype with the difference that the smaller transcript of 1900 nt is less abundant than in the normal fertile cytoplasm.

Discussion

It is becoming increasingly apparent that the cms trait in higher plants is the result of structural mutations in the mitochondrial genomes of fertile plants. Evidence for this are demonstrations of differences in the restriction patterns of mtDNA between fertile and sterile cytoplasms. There are specific differences in the set of restriction fragments in the mtDNAs of fertile and sterile plants of many species including maize (Levings and Pring 1976; Pring and Levings 1978), wheat (Quetier and Vedel 1977; Ricard et al. 1986), sorghum (Conde et al. 1982; Pring et al. 1982; Lee et al. 1989), bean (Boutry and Briquet 1982), sunflower (Leroy et al. 1985; Brown et al. 1986; Crouzillat et al. 1987), rape (Vedel et al. 1982; Erickson et al. 1986), rice (Mignouna et al. 1987), rye (Tudzynski et al. 1986) and millet (Smith et al. 1987). Changes in the structure of mtDNA from sterile plants may be caused not only by rearrangements within the mitochondrial genome, by intra- and intermolecular recombination between the repeats, but also by duplications and/or deletions, as demonstrated for the C group of maize-sterile cytoplasm (Pring et al. 1987) and the bean mitochondrial genome *Phaseolus vulgaris* (Mackenzie et al. 1988) or by insertions, as reported for sunflower *Helianthus annus* (Sicullela and Palmer 1988).

Comparative restriction analyses of mtDNAs from cytoplasms of fertile and sterile sugar beets have been extensively performed (Powling 1982; Powling and Ellis

1983; Mikami et al. 1984, 1985; Duchenne et al. 1989; Dudareva et al. 1990; Komarnitsky et al. 1990; Weihe et al. 1991), and, as a result, significant variations in mitochondrial genome organization between sterile and fertile plants have been revealed.

The important question was whether or not changes in mitochondrial genome organization of sterile sugar beet lines can alter the location of the protein-coding mitochondrial genes? Blot hybridization with the use of the *COXI, COXII, COB,* and *atpA* genes as probes demonstrated that changes in the mitochondrial genome organization of sterile lines of *Beta vulgaris* L. are associated with variations in the location of *COB, atpA,* and *COXII,* but not of *COXI.* No polymorphism for the location of these genes in the mitochondrial genomes of five fertile lines (sterility maintainers, O-type) was found. Using the *COXII* and *atpA* genes as hybridization probes, we revealed differences not only in mtDNAs between fertile and sterile cytoplasms, but also differences in the primary structure of mtDNAs from sterile lines of different origin.

Sugar beet fertile sublines have been identifed that are able to spontaneously convert from the fertile to sterile phenotype. The conversion to sterility was associated with alterations in the location of the *COXII* and *atpA* genes; the mitochondrial genomes of the spontaneous convertants and their sterile counterparts were identical (Dudareva et al. 1990).

It is noteworthy that, of the numerous possible rearrangements in the mitochondrial genome detected in the sterile plants, only a small proportion seems to be causally linked to the cms trait. The mtDNA of sterile radish differs from its fertile counterpart by a minimum of 10 inversions, and only some of these may be relevant to the expression of the cms trait (Makaroff and Palmer 1988). A thoroughly studied case is maize with the Texas (T) type of sterile cytoplasm. In this maize multiple recombination events have given rise to a unique recombination gene, *urf13-T* (Dewey et al. 1986), whose protein product is involved in the determination of the cms trait (Dewey et al. 1986, 1988; Rottmann et al. 1987; Wise et al. 1987 a, b). At least two mitochondrial genome rearrangements gave rise to a novel variant form of the *COXI* gene in sterile 9E cytoplasm of sorghum, and this, in turn, led to an altered transcription and synthesis of a longer subunit I polypeptide of 42 kD replacing the normal 38 kD (Bailey-Serres et al. 1986).

The presence of a chimeric variant of the *Pcf* gene is characteristic of *Petunia* lines with the cms trait: transcription proceeds 4–5 times more intensely in anthers than in leaves of the cms plants (Young and Hanson 1987).

We addressed two key questions in the present study. Firstly, does the mitochondrial genome reorganization we observed in the sterile lines of sugar beet affect the transcription of the protein-coding mitochondrial genes, and, secondly, can the nuclear fertility restorer genes possibly affect the transcription of the mitochondrial genes in plants retaining sterile cytoplasm? To provide answers, by means of Northern hybridization we studied transcription of the mitochondrial genes *COXI, COXII, COB* and *atpA* in fertile and sterile lines of sugar beet and also the possible effect of the nuclear fertility restorer genes on the expression of these genes.

COB was the only mitochondrial gene of those we studied whose transcription was identical in the fertile and sterile cytoplasms, although its location changed. Alterations in transcriptional patterns were observed for the other three mitochondrial genes (*COXI, COXII,* and *atpA*), although transcripts, which are as a rule present in the fertile cytoplasm, occurred in sterile plants. With regard to *COXII* and *atpA,* altered transcription in sterile cytoplasm may presumably be the result of mitochondrial genome reorganization that might have somehow affected these genes. This explanation does not hold true for *COXI* because it is an exception to the other three genes: its location did not change in the sterile cytoplasm. Alterations in the transcription of *COXI* in the mitochondria of sugar beet sterile lines seem to result from either modifications at the initiation (termination) sites of transcription or at some processing stage of RNA.

According to Brears and Lonsdale (1988), who have physically mapped the mitochondrial genome from the Owen-sterile cytoplasm of sugar beet, the *COXI, COB* and *atpA* genes are represented by single copies in the genome. If this is so, the existence of multiple transcripts for these genes cannot be attributed to an increase in copy number. Rather, the transcripts were multiple because of the presence of intermediates formed from a single RNA precursor or because of several initiation or termination sites of transcription arisen within a single gene.

One noteworthy observation is the ability of the nuclear genome to affect the transcription of mtDNA. When dominant nuclear fertility restorer genes were incorporated into plants with sterile cytoplasms, the transcription of certain mitochondrial genes altered. A case in point is the *COB* gene whose transcripts of 3800 nt are present in fertile and sterile cytoplasms and absent from the mitochondria of nucleus-restored fertile plants.

The nuclear *Rf*-genes can restore transcription of the mitochondrial genes: in this case, the transcription of a mitochondrial gene becomes identical to its counterpart with the fertile phenotype irrespective of nuclear background. An example of that is the *atpA* gene whose transcripts are of the same size in plants with genotypes *Nrfrf* and *SRf-*. Thus, the transcription of the *COB* and *atpA* genes is altered depending on the state of the nuclear restorer of fertility genes; in the case of the *atpA* gene it is dependent upon the type of mtDNA, whereas in that

of *COB* it is not dependent upon the type of mtDNA. Possibly, the presence of an *atpA* transcript of 2000 nt, a feature of the sterile phenotype only, is linked to the cms trait. When taking into account that there is no full identity for the nuclear genes in plants with restored fertility and plants with the S- and N- phenotype, the alterations in transcriptional patterns cannot be entirely explained by the effect of the fertility restorer genes. It cannot be ruled out that some other nuclear genes whose effects are, so far, phenotypically undetectable, may affect, along with the fertility restorer genes, the expression of the mitochondrial genes.

The influence of the nuclear fertility restorer genes on the transcription of the mitochondrial genes has been investigated in radish (Makaroff and Palmer 1988) and sunflower (Siculella and Palmer 1988). In both plant species, the nuclear genes can influence the transcription of the gene *atpA;* no altered transcriptional patterns were observed for the other three genes examined, *atp6, COXI* and *COXII.* The question of whether or not alterations in the transcription of the *atpA* gene produces changes in the expression of the cms trait remains open. Alterations in the expression of other mitochondrial genes may be postulated to account for the differences in the cms trait; however, the involvement here of the *atpA* gene appears to be very probable.

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