

## Mitochondrial DNA Variation in Maintainer Lines (O-type) of Sugarbeet

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### ABSTRACT

The mitochondrial genome influences the cytoplasmic male sterile trait in sugarbeet (*Beta vulgaris* L.); therefore, comparisons of mitochondrial DNA structure and organization of sterile and normal cytoplasm can elucidate the molecular basis of male sterility. Mitochondrial DNA variability in maintainer lines of sugarbeet was characterized by restriction fragment length polymorphisms (RFLP). Mitochondrial DNA of four lines from a North American monogerm population and six lines from a Russian monogerm population were restricted with *EcoRI* and hybridized with *coxII* and *atpA* probes. *EcoRI* digestion revealed three distinct restriction patterns: one typical of the pattern for normal cytoplasm of sugarbeet and two that resembled patterns for mtDNA from fertile plants of wild beet, *B. v. ssp. maritima*. The probing of mtDNA with *coxII* revealed two mitochondrial variants differing in a 1.8 kbp hybridization signal. The *atpA* probe identified alterations of mitochondrial DNA which resulted either in the appearance of a new 3.5 kbp band or in the absence of a 4.4 kbp band and the presence of 3.5 kbp signal. Changes in mitochondrial genome organization were observed only in maintainer lines from the Russian population. These differences in mitochondrial DNA provide evidence for the existence of two unique fertile cytoplasm for use in the development of male-fertile sugarbeet breeding lines. RFLP analysis and molecular characterization of mitochondrial DNA of male-fertile germplasm provides breeders useful information on

**genetic diversity within O-type lines. Maintainer lines with unique mtDNA can be used to diversify sugarbeet germplasm.**

**Additional Key Words:** *atpA*, *Beta vulgaris*, *coxII*, mitochondrial genome diversity, RFLP

Cytoplasmic male sterile (CMS) lines are used routinely in the production of commercial sugarbeet hybrids. The male sterile cytoplasm used in Europe and the USA was discovered by Owen. This cytoplasmic uniformity has encouraged studies of physical and gene organization of *Beta* mitochondrial DNA.

Male sterility in sugarbeet sterile lines is associated with mitochondrial DNA rearrangement resulting from recombination and/or duplication of mitochondrial genes and repeated DNA elements. Molecular analysis of mtDNA showed striking differences in the organization of mitochondrial genomes of sterile and fertile cytoplasm. Southern hybridizations of digested mtDNA with *coxII*, *cob*, *atpA* and *atp6* probes confirmed structural differences between mtDNA of fertile and sterile cytoplasm (Duchenne et al., 1989; Senda et al., 1993; Xue et al., 1994).

The mitochondrial diversity in sugarbeet with normal cytoplasm has been studied less than CMS lines. RFLP analysis of mtDNA allows detection of mitochondrial genome variability in maintainer lines (O-type). Relationships among cytoplasm in male-fertile lines and parental populations used for their selection also can be determined by RFLP analysis.

## MATERIALS AND METHODS

Experiments were conducted using line LO6 and three sublines of LO8 indexed for O-type from a monogerm American population and two sublines each from LO9, LO10 and LO12, lines selected from a Russian monogerm population.

Mitochondrial DNA was isolated from roots using the method of Wilson and Chourey (1984). *EcoRI* digestion products were separated on 0.8% agarose gels, transferred to nylon membranes and hybridized for 16 h at 65°C with *coxII* and *atpA* probes. Digoxigenin-dUTP-labeled *atpA* probes were produced in PCR reactions using primers that amplified part of the 5' flanking sequences and a small part (156 bp) of the *atpA* gene for CMS mtDNA of sugarbeet (Xue et al., 1994). Primer pair sequences for the *atpA* probe were:

*atpAa* = 5'-ATCCGCTTGATCTTTCTTTTC

*atpAb* = 5'-CCCAGCTTGAATCTCGTT

The annealing temperature for primers was 54°C.

The *coxII* probe was a 5kb *SacI* fragment containing the *coxII* gene and its flanking sequences isolated from a highly inbred fertile sugarbeet line. A plasmid bearing the gene was kindly provided by Dr. J. Hirshberg (Jerusalem University, Israel). The *coxII* probe was labeled by random primed incorporation of digoxigenin-dUTP. Immunodetection was performed with an anti-digoxigenin-alkaline phosphatase antibody and reactivity was visualized by CSPD on X-ray films.

## RESULTS

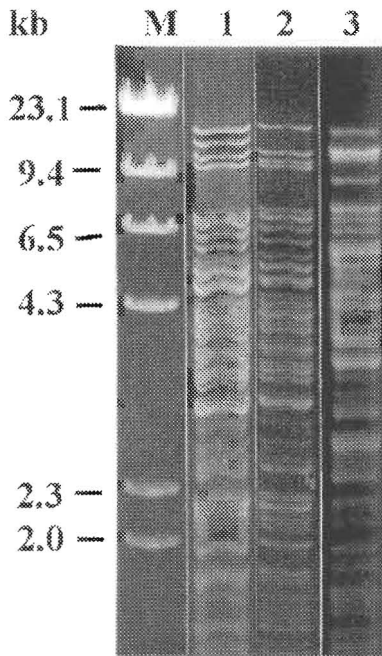
The digestion of mtDNA with *EcoRI* revealed three restriction patterns. Lines LO6 and LO8 possessed a restriction pattern typical of normal (N) cytoplasm of sugarbeet. This pattern was characterized by the occurrence of four specific fragments (18.4, 15.9, 13.0 and 11.3 kbp) in the top of the gel (Fig. 1A). Mitochondrial DNA of LO9 lines contained an additional 14.0 kbp band and lacked the 15.9 kbp band. The restriction pattern for LO10 and LO12 did not have a 15.9 kbp band but contained an 8.6 kbp fragment.

The alterations in *EcoRI* restricted mtDNA found in some maintainer lines were associated with differences in organization of *coxII* and *atpA* genes. Probing of mtDNA with *coxII* produced an additional hybridization signal (1.8 kbp band) for LO9A, indicative of a novel cytoplasm (Fig. 1B). Hybridization of *EcoRI* mtDNA fragments with an *atpA* probe revealed: 1) the appearance of a new 3.5 kbp band for LO9A, 2) the absence of 4.4 kbp band and the presence of additional signal (3.5 kbp) in the hybridization fragments for LO10A, LO10B, LO12 and LO12F, lines possessing altered mitochondrial genomes (Fig. 1C).

## DISCUSSION

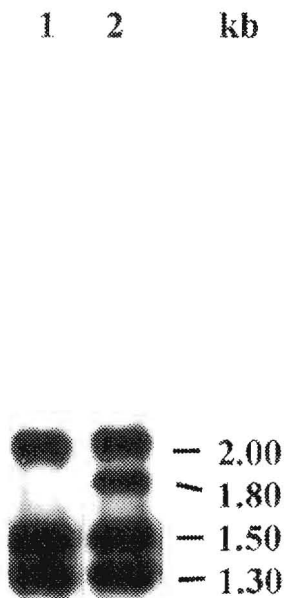
The CMS trait in sugarbeet is associated with highly rearranged mitochondrial genomes. Searches for male-sterile sources that could provide an alternative to the Owen CMS source revealed either subtypes of mitochondrial genomes within CMS breeding lines or unique sterile cytoplasms in wild beet, mainly in *B.v. ssp. maritima* (Weihe et al., 1991; Boutin et al., 1987).

Information regarding diversity within normal cytoplasms is limited. We analyzed four maintainer lines (O-type) from a North American monogerm population and six lines from a Russian monogerm population. The *EcoRI* digestion of mtDNA discriminated different restriction patterns. Two resembled patterns from mtDNA of fertile plants of wild beet digested with *EcoRI* and classified as N<sub>2</sub> and N<sub>3</sub> types of *B.v. ssp. maritima* cytoplasm (Saumittou-Laprade et al., 1993).

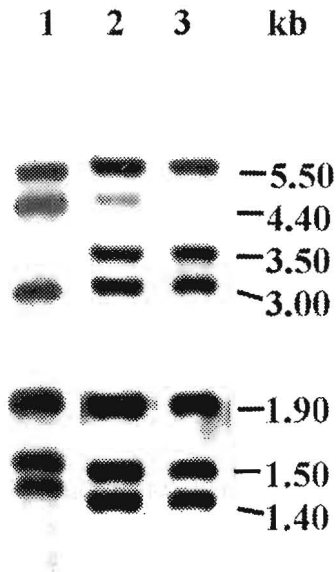


**Fig. 1A.** The variability of mitochondrial DNA (mtDNA) within male-fertile (O-type) sugarbeet lines. *Eco*RI restriction patterns of mtDNA for LO6, LO8E, LO8F and LO8J (lane 1); LO9J and LO9A (lane 2); and LO10A, LO10B, LO12 and LO12F (lane 3).

Lambda DNA-*Hind*III restriction fragments were used as molecular weight markers (M lane).



**Fig. 1B.** The variability of mitochondrial DNA (mtDNA) within male-fertile (O-type) sugarbeet lines. Hybridization profiles for mtDNA probed with *coxII*. Lane 1: the profile for LO6, LO8E, LO8F, LO8J, LO9J, LO10A, LO10B, LO12 and LO12F; lane 2: the profile for line LO9. Lambda DNA-*HindIII* restriction fragments were used as molecular weight markers (M lane).



**Fig. 1C.** The variability of mitochondrial DNA (mtDNA) within male-fertile (O-type) sugarbeet lines. Hybridization profiles for mtDNA probed with *atpA*. Lane 1: the profile for LO6, LO8E, LO8F, LO8J and LO9J; lane 2: the profile for LO9A; lane 3: the profile for LO10A, LO10B, LO12 and LO12F.

Lambda DNA-*Hind*III restriction fragments were used as molecular weight markers (M lane).

A new fertile mitochondrial genome slightly different from the genome characteristic of normal cytoplasm was found by Duchenne et al. (1989); however, the *EcoRI* restriction pattern of this line did not resemble patterns for fertile plants of *B.v. ssp. maritima*. Komarnitsky et al. (1990) showed that *BamHI*-digested mtDNA of several maintainer lines differed from mtDNA of their respective fertile progenitors.

The diversity of mtDNA within maintainer lines occurred within the Russian monogerm population, according to the results of this study. The similarity of two maintainer cytoplasmic types to the fertile cytoplasm identified in *B.v. ssp. maritima* could indicate that the fertile cytoplasm of cultivated sugarbeet originated from *B.v. ssp. maritima*. On the basis of the identical mitochondrial RFLP profiles for fertile sugarbeet (*B.v. ssp. vulgaris*) and *B.v. ssp. maritima* obtained with *coxI*, *coxII* and *atpA* probes, Senda et al. (1995) confirmed a phylogenetic relationship between these species and their classification into the section *Beta*.

Observed changes within male fertile lines detected by *EcoRI* digestion of mtDNA, were confirmed by Southern hybridization with *coxII* and *atpA* probes. The distinct changes in *atpA* and *coxII* hybridization patterns within maintainer lines are the first report of mitochondrial variability revealed by *atpA* and *coxII* probes. Duchenne et al. (1989) detected rearrangement for only the *26S* gene in a fertile variety with unique *EcoRI* restriction patterns; whereas, *coxII* and *atpA* probes did not indicate any mitochondrial alterations.

The occurrence of polymorphic bands in hybridization profiles for both probes can result from changes of *EcoRI* restriction sites and/or organization of *coxII* and *atpA* loci. Because both probes used in our studies contained the large 5' flanking sequence that allowed detection of changes within those loci, the probes were more informative and specific than those containing only exons of *coxII* or *atpA* coding sequences (Mann et al., 1991; Sadoch and Goc, 1997).

Differences in the organization of mtDNA, as shown by RFLP analyses, revealed mitochondrial genome diversity within male-fertile breeding (O-type) lines. Our results indicate substantial variability within fertile cytoplasm of sugarbeet. RFLP analysis and molecular characterization of mitochondrial DNA of male-fertile germplasm provides breeders with useful information on genetic diversity within O-type lines, based on mitochondrial genome differences and not solely on nuclear DNA polymorphism. Maintainer lines with unique mtDNA can be used to diversify sugarbeet germplasm in breeding programs.

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### ACKNOWLEDGMENTS

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