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A new cytoplasmic male sterile genotype in the sugar beet *Beta vulgaris* L.: a molecular analysis

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Summary. Mitochondrial DNA (mtDNA) from fertile (N) and possibly new cytoplasmic male sterile (CMS) genotypes was studied in the sugar beet *Beta vulgaris* L. It was found by restriction endonuclease analysis that BMC-CMS, a cytoplasm that was derived from the wild beet *Beta maritima*, contained a unique type of mtDNA which is distinguishable from both the N and S-CMS, the only other CMS genotype that is currently availabe in *B. vulgaris* L. The organization of three genes: *coxI*, *coxII* and *cob*, was analyzed by hybridization with heterologous probes from maize. These genes have a similar structure in N and BMC-CMS that is different from S-CMS. It is concluded that BMC-CMS is a novel CMS genotype in the sugar beet.

Key words: Cytoplasmic male sterility – Mitochondrial DNA – Sugar beet

Introduction

Cytoplasmic male sterility (CMS) in the sugar beet *Beta vulgaris* L. was discovered and studied by Owen in the 1940s (Owen 1942, 1945). This type of CMS, called S or "Owen's type", is currently the only cytoplasm that is being used worldwide for the production of hybrid varieties of sugar beet. As in other species, the sterile phenotype is a result of an interaction between a cytoplasmic (mitochondrial) factor(s) in combination with nuclear genes termed X and Z (Owen 1945; Owen 1950).

Several lines of evidence suggest that CMS is encoded by the mitochondrial genome (reviewed in Londsdale 1987). The most direct one comes from somatic hybridization experiments where CMS was shown to genetically segregate independent of plastids (Fluhr et al. 1983; Clark et al. 1985).

The mitochondrial genome of sugar beet consists of a high molecular weight (HMW) DNA (ca 400 kb) (Powling 1982), as well as small supercoiled circular molecules (Powling 1981; Powling and Ellis 1983). Comparison of mitochondrial DNA (mtDNA) from fertile and CMS lines revealed differences in the pattern of restriction enzyme cleavage of the HMW DNA as well as in the number and type of the small supercoiled molecules (Powling 1981, 1982; Powling and Ellis 1983; Mikami et al. 1984, 1986; Hallden et al. 1988). Less variation exists between chloroplast genomes from comparable cytoplasms (Powling and Ellis 1983; Mikami et al. 1984, 1985; Kishima et al. 1987; Hallden et al. 1988).

A survey of mitochondrial genomes from Oldemeyer's CMS collection, derived from wild beets and constructed in an isogenic nuclear background, revealed four groups of CMS genomes characterized by their DNA digestion pattern (Mikami et al. 1985). These CMS types showed three different combinations of mincircular DNA molecules (Mikami et al. 1986). Three of the minicircles were studied extensively by Hansen and Marcker (1984) and Thomas (1986). However, as pointed out by Hallden et al. (1988), it is possible that the whole phenomenon is simply co-inherited with CMS and does not relate to it at all.

Our current knowledge about the molecular basis of CMS comes mainly from studies done in maize (Dewey et al. 1988) and Petunia (Young and Hanson 1987). In these cases CMS is associated with mtDNA rearrangements, which result in expression of chimeric genes whose protein products are thought to interfere in yet unknown mechanism(s) with normal pollen development (reviewed by Londsdale 1987). In an attempt to understand the molecular basis of CMS in sugar beet, and in

a search for new types of CMS, we have studied the organization of the mitochondrial genome of fertile (N) and different novel types of CMS lines.

We report here the characterization of the high molecular weight (HMW) mtDNA from N (normal), S-CMS (Owen's type), and isolines of new types of male sterile cytoplasms: BMC, introduced originally from *Beta maritima* (Coe and Stewart 1977), and S_{i-2}, S_{i-3} and S_{i-4} isolated from N following gamma irradiation (Kinoshita 1977). Our results indicate that BMC is a novel type of CMS, distinct from Owen's S-type, while the other CMS types have the same characteristics as S-CMS.

Materials and methods

Plant material

Seed for all genotypes of sugar beet *Beta vulgaris* L. used in this study were from USDA-ARS sources at East Lansing, Michigan. The normal (N) fertile cytoplasm and S-CMS (Owen's type) sterile cytoplasm were from the highly inbred line NB-1. S_{i-2}, S_{i-3} and S_{i-4} are new CMS types derived from the Japanese N cytoplasm variety, H2002, following gamma irradiation, by Kinoshita (1977). Seeds were kindly supplied to C. Theurer by Dr. Kinoshita. BMC-CMS sterile cytoplasm was derived from male sterile plants found in an accession of *Beta maritima* collected in 1955 at Wembury Bay near Plymouth, England, and introduced into *Beta vulgaris* by backcrossing (Coe and Stewart 1977). The S_{i-2}-CMS, S_{i-3}-CMS, S_{i-4}-CMS and BMC-CMS cytoplasm sources were nearly isogenic for NB-1 nuclear genes having been backcrossed to the NB-1 normal inbred for four generations.

DNA analysis

For extraction of mtDNA, mitochondria were isolated from root tissue following conventional procedures described by Leaver et al. (1983) and mtDNA was extracted and purified as described by Sparks and Dale (1980). Restriction enzyme analysis, agarose gel electrophoresis, and Southern hybridization were carried out according to Maniatis et al. (1982).

Results

Restriction patterns of mtDNA

Mitochondrial DNA was extracted from N and various CMS cytoplasms, cut with several restriction enzymes, and the resulting fragments were separated by agarose gel electrophoresis. Characteristic and unique restriction pattern are exhibited by each mtDNA of the N, S-CMS and BMC-CMS (Fig. 1). The differences between N and S-CMS are similar to those which have been previously described (Powling 1982). However, the restriction pattern of BMC-CMS is different and distinguishable in a number of bands from both N and S-CMS. This finding indicates that each of the lines contains a different type of mitochondrial genome.

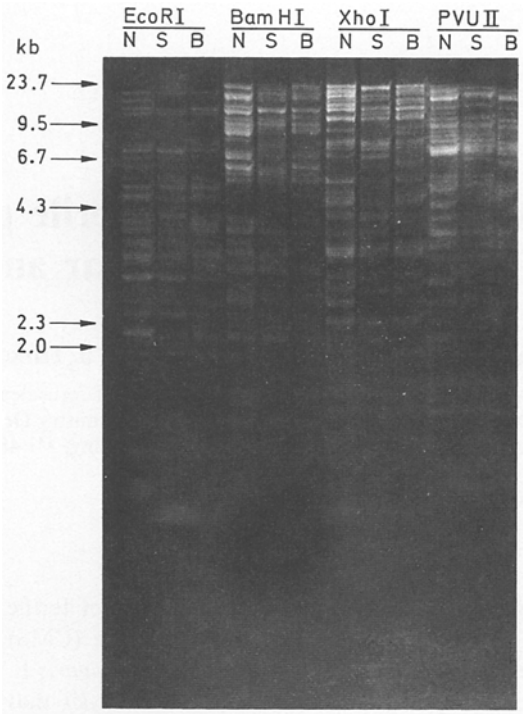

Fig. 1. Restriction endonuclease analysis of mtDNAs. Ethidium bromide (EtBr)-stained agarose gel of mtDNA from normal (N), S-CMS (S), and BMC-CMS (B), digested with the restriction enzymes EcoRI, BamHI, XhoI, and PvuII. Fragment sizes are indicated in kilobase pairs (kb)

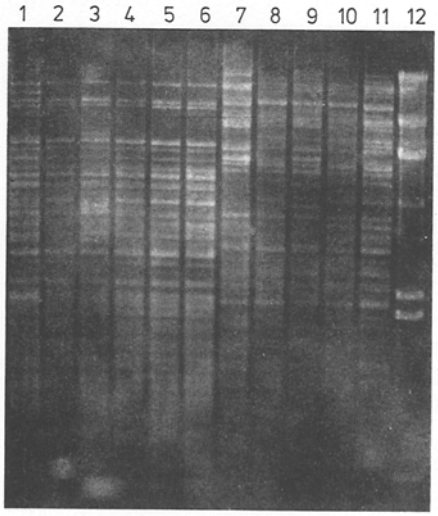

Fig. 2. Restriction endonuclease analysis of mtDNAs. Ethidium bromide-stained agarose gel of mtDNAs from fertile – N (*lane 1*), S-CMS (*lane 2*), BMC-CMS (*lane 3*), S_{i-2}-CMS (*lane 4*), S_{i-4}-CMS (*lane 5*), S_{i-3}-CMS (*lane 6*), digested with EcoRI. N (*lane 7*), S-CMS (*lane 8*), BMC-CMS (*lane 9*), S_{i-2}-CMS (*lane 10*), S_{i-4}-CMS (*lane 11*), digested with BamHI. Lambda DNA digested with HindIII serves as size marker (*lane 12*)

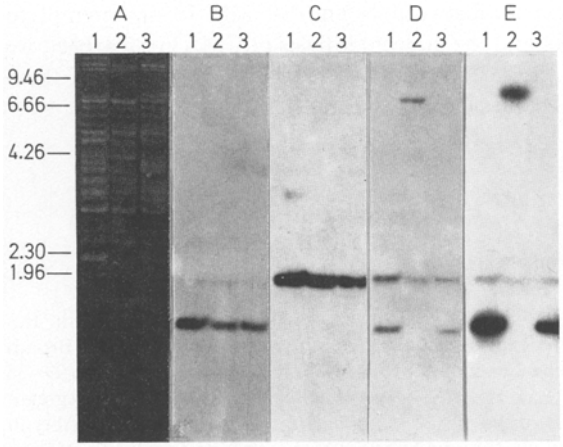

Fig. 3A–E. Southern hybridization analysis of mtDNA from the fertile and CMS lines of sugar beet. mtDNA from fertile – N (1), S-CMS (2), and BMC-CMS (3) were cut with the restriction enzyme EcoRI and separated by agarose gel electrophoresis. **A** shows the restriction pattern after EtBr staining of the gel. The gel was blotted to a nitrocellulose filter and hybridized with various ^{32}P-labelled DNA probes and autoradiographed. The probes were: **B** – *coxI* of maize; **C** – *cob* of maize; **D** – *coxII* of maize; **E** – the cloned 1.6-kb EcoRI fragment of mtDNA from N sugar beet, which is homologous to *coxII* (see text). Fragment sizes are indicated in kb

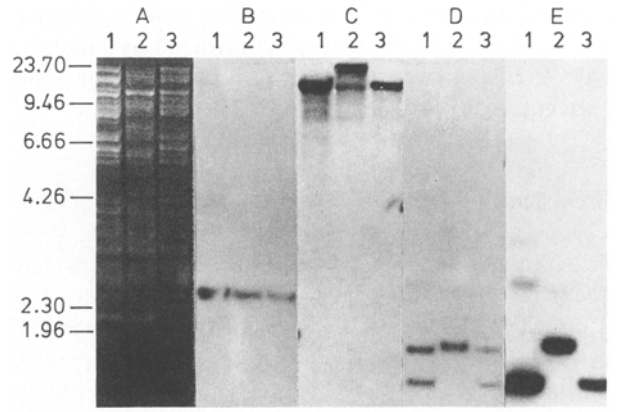

Fig. 4A–E. Southern hybridization of mtDNA from fertile and CMS lines of sugar beet. Analysis was exactly as described in Fig. 3, using the same DNAs and probes, but DNAs were cleaved with the restriction enzyme BamHI. Fragment sizes are indicated in kb

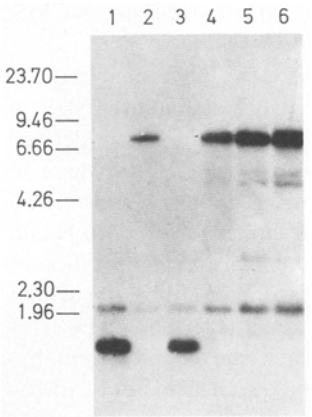

Fig. 5. Southern hybridization of mtDNAs from fertile – N (1), S-CMS (2), BMC-CMS (3), S_{i-2}-CMS (4), S_{i-4}-CMS (5) and S_{i-3}-CMS (6). mtDNAs were digested with EcoRI, separated by an agarose gel electrophoresis, and blotted to a nitrocellulose filter. Hybridization was done with the 1.6-kb DNA fragment of N mtDNA from sugar beet containing *coxII*-homologous sequence. Fragment sizes are indicated in kb

Similar analysis was carried out with three CMS mutants that were isolated from N, following gamma irradiation (Kinoshita 1977). As can be seen in Fig. 2, mutants S_{i-2}, S_{i-3}, and S_{i-4} have exactly the same mtDNA restriction pattern as the S-CMS when cleaved with EcoRI or BamHI. The same result was also obtained when the mtDNAs were analyzed with the restriction enzymes SalI and HindIII (data not shown).

Genes organization in the mitochondrial genome

In order to further analyze mtDNA organization in the various lines, we have carried out a Southern hybridization analysis with cloned mitochondrial genes from maize as heterologous probes. The genes used were: *coxI* and *coxII,* coding for subunits I and II of cytochrome oxidase (Isaac et al. 1985; Fox and Leaver 1981), and *cob,* coding for apocytochrom B (Dawson et al. 1984) (kindly given to us by Dr. C. Leaver). The results (Figs. 3 and 4) show that *coxII* hybridized to DNA fragments of different sizes in S-CMS as compared to N and BMC-CMS, when digested with EcoRI or BamHI restriction endonucleases. As can be seen in Fig. 3D, two EcoRI fragments of 1.6 kb and 2.0 kb from N mtDNA hybridized with maize *coxII*. The 1.6-kb fragment was cloned in the *E. coli* plasmid pUC118 and used as a DNA probe in Southern hybridization analysis of the same restricted DNAs (Figs. 3E, 4E). Different hybridization patterns of *coxII* to mtDNA from N and S-CMS were found after digestion with either EcoRI or BamHI. These results further emphasize the differences in genome organization between N and S-CMS. However, it is clearly demonstrated that the hybridization pattern of the three genes in BMC-CMS is identical to N (the normal type).

Southern hybridization of *cob* to mtDNA digested with BamHI revealed a different hybridization pattern in S-CMS as compared to N and BMC-CMS (Fig. 4C). However, since no difference was detected in the EcoRI cleavage (Fig. 3C), it is possible that this difference reflects a polymorphism in DNA sequence of the EcoRI restriction site.

Mitochondrial DNA from S_{i-2}-CMS, S_{i-3}-CMS and S_{i-4}-CMS was examined by Southern hybridization

as above, using the 1.6-kb fragment containing the sugar beet *coxII* as a probe. The results shown in Fig. 5 indicate that *coxII* in these genotypes is located on the same fragments as in S-CMS.

Discussion

The Texas male-sterile cytoplasm (CMS-T) was widely used in commercial production of hybrid maize varieties before severe disease outbreaks in 1970 forced the industry to limit its use. CMS-T is unique in that an inseparable association appears to exist between disease susceptibility to the fungal pathogen *Bipolaris* (*Helminthosporium*) *maydis* race T, and male sterility in this cytoplasm (Hooker et al. 1970). Sugar beet is considered vulnerable to pathogens and stress because of the uniformity and narrowness of its genetic background (Coe and Stewart 1977; Bosemark 1979). All the hybrid varieties currently in agricultural use carry the same cytoplasm, S-CMS, which has been found by Owen (1945).

In the study presented here, we have analyzed the high molecular weight mtDNA of possible new types of sugar beet CMS. The BMC-CMS, which was derived from the wild beet *Beta maritima,* was shown here to contain a unique mitochondrial genome which is different in the pattern restriction fragments from both N and S-CMS. In spite of these differences, the organization of its mitochondrial genome regarding the three genes that have been examined is identical to the fertile N-type. This is in contrast to S-CMS, which shows a different hybridization pattern of *coxII* and *cob*. In crosses of BMC-CMS with "O-type" (maintainer line) or restorer genotypes of Owen's type, the segregation patterns of sterility apear to be different than in crosses with S-CMS, thus suggesting that the molecular basis for male sterility in BMC-CMS is probably different than that of S-CMS. This conclusion makes BMC-CMS very interesting from both academic and breeding points of view as a potential alternative for S-CMS.

The CMS mutants, S_{i-2}, S_{i-3}, and S_{i-4}, which have been derived from the N-type, are shown here to contain high molecular weight mtDNA that is identical to the S-CMS, both in the overall restriction pattern and in the location of specific genes that were examined. These findings could indicate either that the mutagenic event which had led to sterility also induced all the characteristic mtDNA rearrangements that are found in the S-CMS, or that these lines were actually derived from S-CMS.

In two well-studied cases of CMS in maize (Dewey et al. 1988) and in Petunia (Young and Hanson 1987), it has been shown that CMS is strongly correlated with specific mitochondrial genome rearrangements that involve specific genes. In sugar beet we also find that *coxII* and *atp6* (data not shown) are involved in mtDNA rearrangements between N and S-CMS. In an attempt to understand the molecular basis of CMS in sugarbeet, we continue to look for differences in expression of these genes between S-CMS and N.

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