BARD

FINAL REPORT

PROJECT NO. I-788-84

The Molecular Basis of Cytoplasmic Male
Sterility in Sugar-Beet (*Beta Vulgaris* L.)

J. Hirschberg, L. McIntosh, C. J. Theurer

1989

Standard BARD Cover Page for Scientific Reports

Date: June 1989

BARD
P.O. BOX 6
Bet Dagan, ISRAEL

29-8-1989

BARD project No. I - 788 - 84

Title

The molecular basis of cytoplasmic male sterility in the sugar beet

Beta vulgaris L.

Investigators' Names
(Principal listed first)

Joseph Hirschberg

Lee McIntosh

Clair Theurer

Investigators' Institutions

The Hebrew University of Jerusalem

Michigan State University

USDA ARS East Lansing

Project's starting date: October 1985

Type of Report: 1st Annual 2nd Annual Final X

Joseph Hirschberg
Signature
Principal Investigator

Y. Haas
Signature
Institution's Authorizing Official

Tabel of contents:

	<u>Page</u>
Abstract	3
Objectives of the original research.....	4
Body of the report:	
Introduction.....	5
Materials and methods.....	7
Results:	
BMC-CMS - a stable CMS genotype.....	9
Restriction patterns of mtDNA.....	10
Gene organization in the mitochondrial genome.....	12
Gene expression in mitochondria - RNA synthesis.....	19
Gene expression in mitochondria - protein synthesis.....	26
Molecular analysis of <u>coxII</u> gene.....	30
Discussion.....	35
References.....	38
Description of cooperation.....	40
Evaluation of research achievements.....	41
Publications.....	42

ABSTRACT

A novel cytoplasmic male sterility (CMS) has been identified in the sugar beet Beta vulgaris L. Mitochondrial DNA (mtDNA) from fertile (N) and cytoplasmic male sterile (CMS) genotypes of sugar beet. 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 available in B. vulgaris L. The organization of five genes: coxI, coxII, atp6, atp9 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.

Gene expression in the mitochondria was studied in the fertile and the S-CMS genotypes. Only minor differences were observed in polypeptides that were synthesized in organello. Qualitative and quantitative differences in transcription of the genes coxII and atp6 were observed between the fertile and S-CMS. The connection between these differences and CMS is unclear.

The gene coxII was cloned from fertile, S-CMS and BMC-CMS. Its nucleotide sequence was determined for the first exon and almost the entire intron. The amino acid sequence of subunit II of cytochrome oxidase in sugar beet is 84% similar to the gene in maize and 94% similar to the soybean gene. No differences were found between N and BMC-CMS. An additional DNA sequence that is homologous to part of coxII was identified in the mtDNA of all genotypes of the sugar beet.

OBJECTIVES OF THE ORIGINAL RESEARCH PROPOSAL

The original proposal was aimed at studying cytoplasmic male sterility (CMS) in the sugar beet focusing on two unique phenomena related to CMS which exist in this species. These are: a) The novel CMS types which were isolated in Japan from the fertile cytoplasm by gamma irradiation and in the USDA by outcrossing B. vulgaris with B. maritima; b) The "intermediate sterility" cases described in sugar beet.

The objective was to analyze the mitochondrial genome, its overall structure and gene organization, as well as gene expression, in the sugar beet in order to correlate a molecular observation with the CMS phenotype. A similar approach that has been taken for studying CMS in other species was successful in establishing such correlations, however a clear cut causal relationship between the molecular findings and CMS has not been demonstrated.

INTRODUCTION

The overall objective of this research was to investigate the molecular basis of cytoplasmic male sterility (CMS) in the sugar beet (Beta vulgaris L.). Since this trait was shown in different species to be encoded by the mitochondrial genome (chondriome) we have focused our study on the sugar beet chondriome, its structure, gene organization and gene expression, in fertile (N) and different male sterile genotypes.

Cytoplasmic male sterility (CMS) in the sugar beet Beta vulgaris L. was discovered and studied by Owen in the 1940's [1,2]. 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 [3].

Several lines of evidence suggest that CMS is encoded by the mitochondrial genome [reviewed in 4]. The most direct one comes from somatic hybridization experiments where CMS was shown to genetically segregate independent of plastids [5,6].

The mitochondrial genome of sugar beet consists of a high molecular weight (HMW) DNA (ca.400 kb) [7] as well as small supercoiled circular molecules [8,9]. 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 [7-9]. Less variation exists between chloroplast genomes from comparable cytoplasms [9,11-13].

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 [12]. These CMS types showed three different combinations of minicircular DNA molecules [10]. Three of the minicircles were studied extensively by Hansen and Marcker [14] and Thomas [15]. However, as pointed out by Hallden et al. [11], it is possible that the whole phenomenon is simply coinherited 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 [16] and Petunia [17]. 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 in 4]. 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 and gene expression of the mitochondrial genome in fertile (N) and different novel types of CMS lines.

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. Si-2, Si-3 and Si-4 are new CMS types derived from the Japanese N cytoplasm variety H2002, following gamma irradiation, by Kinoshita [18]. 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 [19]. The Si-2-CMS, Si-3-CMS, Si-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.

Two isolines series of sugar beet required for the research has been prepared by Dr. C. Theurer:

- a) L29 series - This varieties contain the Owen's type of CMS-CMS-US1: L29 CMS; L29 "o-type" normal; L29(N) Rf (L29 CMS restored with a L29 pollen restorer having normal cytoplasm in the restorer); and L29 (S) Rf - (an L29 pollen restorer having sterile cytoplasm and restorer genes in the pollinator).
- b) Beta maritima series - BMC-CMS, male sterile derived from a segregating generation of Beta maritima male sterile, crossed

to a sugar beet "O-type"; BMC Rf, (CMS-BMC bc4 to L60 Rf); BM-CMS, (a cytoplasmic male sterile from Beta maritima X SP73550-0 sugrbeet); BM Normal (an "O-type" normal pollinator equal to SP73550-0).

Following is a list of lines:

BMC-CMS, BMC cytoplasm bc4 to Cl;
BMC-Rf, BMC cytoplasm Rf1 Rf2 genes;
BM-CMS, male sterile cytoplasm from B. maritima -
Beltsville source;
BM-(N), "O-type" - normal cytoplasm non-restorer genes
-equivalent to BM-BMC;
Cl(N), normal cytoplasm non restorer genes - equivalent
of Owen's type;
L29(N), normal cytoplasm non restorer genes - equivalent
"o-type" for L29-CMS (Owen's source);
L29-CMS, Owen's CMS in L29 genetic background;
L29(N)Rf, normal cytoplasm, Rf1 Rf2 genes;
L29(S)RF, US1 (Owen's) cytoplasm with Rf1 Rf2 genes;
BMC-CMS-progenitor, 2 generations progenitor of BMC-CMS.

Molecular analyses

For extraction of mtDNA, mitochondria were isolated from root tissue following conventional procedures described by Leaver et al. [20] and mtDNA was extracted and purified as described by Sparks and Dale [21]. Restriction enzyme analysis, agarose gel electrophoresis and Southern hybridization were carried out according to Maniatis et

al. [22]. Mitochondrial RNA extraction, and in organello protein synthesis were according to [20]. "Northern" analysis of RNA was according to Maniatis et al.

RESULTS

BMC-CMS is a stable cytoplasmic male sterile genotype

Development of homozygous CMS and Rf isolines for several sources of CMS has been done by backcrossing to obtain bc6 generations. (We began studies with bc4 material). In addition, clones have been developed, by a short culture technique, for several potentially different sources of CMS in order to maintain genotypes, study their stability under different environmental conditions, determine their genetic response when crossed with CMS-US1-type pollen restorer genes, and the development of isolines for possible future molecular study.

Plants of BMC-CMS which were to be used to make the bc5 generation backcross in 1986 had pale yellow non-dehiscent anthers, instead of the expected white shrunken anther expected. Yellow anthers indicate a tendency towards semi-fertility. In order to find a possible reason for this phenomenon, clones were made of two of these plants and the effect of temperature was studied in the growth chambers under different temperatures: 18°, 24°, and 28° C.

It was found, that regardless of temperature, all clones showed the typical white anther phenotype. No yellow anthers were observed, as previously seen in the greenhouse.

Restriction patterns of mtDNA

Mitochondrial DNA (mtDNA) 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 patterns were 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 [7]. 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.

Similar analysis was carried out with three CMS mutants that were isolated from N following gamma irradiation [18]. As can be seen in Fig. 2, mutants Si-2, Si-3 and Si-4, have exactly the same mtDNA restriction pattern as the S-CMS when cleaved with EcoRI or BamHI. The same result was obtained also when the mtDNAs were analyzed with the restriction enzymes SalI and HindIII (data not shown).

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); Si-2-CMS (lane 4); Si-4-CMS (lane 5); Si-3-CMS (lane 6), digested with EcoRI. N (lane 7); S-CMS (lane 8); BMC-CMS (lane 9); Si-2-CMS (lane 10); Si-4-CMS (lane 11), digested with BamHI. Lambda DNA digested with HindIII serves as size marker.

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 [23,24], and cob, coding for apocytochrom B [25] (kindly given to us by Dr. C. Leaver). The results (Fig. 3-4) 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 1.5 kb and 1.95 kb from N mtDNA, hybridized with maize coxII.

The 1.5 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 (Fig. 3E, 4E). Different hybridization patterns of coxII to mtDNA from N and S-CMS, cleaved with several different restriction enzymes, indicate that these differences result from genome rearrangements that occurred close to coxII in S-CMS rather than from polymorphism in restriction sites. 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).

Fig. 3: Southern hybridization analysis of mtDNA from the fertile and CMS lines of sugar beet. mtDNAs from fertile-N (1), S-CMS (2) and BMC-CMS(3) were cut with the restriction enzyme EcoRI and separated by agarose gel electrophoresis. Panel 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: Panel B - coxI of maize (23); Panel C - cob of maize (25); Panel D - cox II of maize (24); Panel E - the cloned 1.5kb EcoRI fragment of mtDNA from N sugar beet, which is homologous to coxII (see text). Fragment sizes are indicated in kb.

Fig. 4: Southern hybridization of mtDNA from fertile and CMS lines of sugar beet. Analysis was exactly as described in figure 3, using the same DNAs and probes but DNAs were cleaved with the restriction enzyme BamHI.

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 Si-2-CMS, Si-3-CMS and Si-4-CMS was examined by Southern hybridization as above, using the 1.5 kb fragment, containing the sugar beet coxII, as a probe. The results shown in figure 5 indicate that coxII in these genotypes is located on the same fragments as in S-CMS.

We have continued to analyse genome organization in fertile and CMS varieties by analyzing the genes atp6 and atp9. Southern hybridization of total mtDNA isolated from roots of different lines was carried out with heterologouse probes of maize genes (Fig. 6). The results show different sizes of fragments that hybridize to each of the probes in fertile (normal) and S-CMS mitochondria.

Fig. 5: Southern hybridization of mtDNAs from fertile-N (1), S-CMS (2), BMC-CMS (3), Si-2-CMS (4), Si-4-CMS (5) and Si-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 1.5 kb DNA fragment of N mtDNA from sugar beet containing coxII-homologouse sequence. Fragment sizes are indicated in kb.

Fig. 6: Southern blots of sugar beet root mtDNA probed with three maize genes. Sugar beet root mitochondrial DNA was digested with BamH1 endonuclease, electrophoresed, and blotted to nitrocellulose. The radioactively- labelled probes used were: lanes 1-3, maize atp6 gene (29); lanes 4-6, maize atp9 gene (28); lanes 7-9, maize coxI gene (23). The mitochondrial DNA used was: lanes 1,4, & 7, Si-4-CMS mtDNA; lanes 2,5, & 8 C1 normal mtDNA; lanes 3,6, & 9, NB1 mtDNA (a non-induced sterile cultivar).

Gene expression in the mitochondria: RNA synthesis

In order to detect changes in gene expression that might be related to CMS, the mitochondrial mRNA was analysed by hybridization to specific DNA probes (Northern hybridization). Total RNA was extracted from intact isolated root mitochondria, separated by gel electrophoresis and transferred to nitrocellulose filters. Radioactively labelled DNA of the genes coxII, atp6, and atp9, cloned from sugar beet or from maize, was prepared and hybridized to the filters (figures 7-9). Our previous results have indicated a genome rearrangement near the 5'-coding sequence of the cytochrome oxidase, subunit II (coxII) gene in S-CMS of the sugar beet when compared to fertile. The results shown in Fig. 7, indicate that there is a second, larger by about 300 nucleotides, transcript of coxII in the S-CMS mitochondria and also in CMS that had been restored to fertility. The transcripts of atp9 appear similar in all types tested (CMS and normal) while the transcripts of atp6 appear either unstable and/or present at low amounts in CMS and restored-CMS tissues from roots.

Fig. 7: Northern blot of sugar beet root mtRNA probed with Si-4 CMS coxII gene fragment. Mitochondrial RNA was isoalted from sugar beet roots, electrophoresed, and blotted to nitrocellulose. The radioactively-labelled probe used was the 1860 bp fragment of the coxII gene cloned from Si4 CMS beets. The lanes of the ethidium bromide-stained gel (left) correspond to the same numbered lanes in the autoradiograph (right). Lane 1: C1 normal mtRNA; lane 2: S-CMS mtRNA; lane 3: Si-4 CMS mtRNA; lane 4: Si-4 Rf mtRNA. Size standards on the gel are the 23S and 16S rRNA from E. coli

Fig. 8: Northern blot of sugar beet root mtRNA probed with maize atp6 gene. Mitochondrial RNA was isolated from sugar beet roots, electrophoresed, and blotted to nitrocellulose. The radioactively-labelled probe used was the maize atp6 gene (29). The lanes of the ethidium bromide-stained gel (left) correspond to the same numbered lanes of the autoradiograph (right). Lane 1: C1 normal mtRNA; lane 2: S-CMS mtRNA; lane 3: Si-4 CMS mtRNA; lane 4: Si-4 Rf mtRNA. Size standards on the gel are the 23S and 16S rRNA from E. coli.

ATP 9

1 2 3 4

Fig. 9: Northern blot of sugar beet root mtRNA probed with maize atp9 gene. Mitochondrial RNA was isolated from sugar beet roots, electrophoresed, and blotted to nitrocellulose. The radioactively-labelled probe used was the maize atp9 gene (28). Lane 1: C1 normal mtRNA; lane 2: S-CMS mtRNA; lane 3: Si4 CMS-mtRNA; lane 4: Si4 Rf mtRNA.

Cytoplasmic male sterility is manifested phenotypically in the anthers and is assumed to be due to mitochondrial malfunction. It is possible that the defect causing CMS is expressed in this tissue only. In Petunia, increased expression of a CMS-specific gene, that is involved in DNA rearrangement, was found in the anthers [17]. We therefore measured the relative amounts of mRNA of 4 genes in the male floral tissues in order to see if there are changes in the steady state levels of mRNA in sterile tissues relative to fertile ones, which might correlate with CMS. We also wanted to observe the mRNA levels of these genes as part of the normal developmental sequence from buds to anthers to pollen.

Total RNA was extracted from C1 fertile (N) and C1 CMS (S-CMS) tissues and used in "slot blotting" experiments. A developmental down-regulation from buds to anthers to pollen was observed using all four gene probes. Comparing similar tissues in fertile and sterile plants revealed that the expression levels of atp9 (Fig. 10) and atp6 and coxI (data not shown) appeared to be similar. However, the levels of coxII mRNA (Fig. 11), appear to be slightly increased in the S-CMS relative to their fertile counterparts. Whether this phenomenon is related to CMS is not known.

Fig. 10: Measurement of relative amounts of mRNA of the gene *atp9* in mitochondria of fertile (N) and S-CMS (C1 CMS). Total mitochondrial RNA was extracted from different tissues. Similar amounts of RNA at 3 different concentrations were "slot blotted" to a filter. ^{32}P -labelled DNA of *atp9* from maize was hybridized to the filter and autoradiographed.

Fig. 11: Measurement of relative amounts of mRNA of the gene *coxII* in mitochondria of fertile (N) and S-CMS (C1 CMS). Total mitochondrial RNA was extracted from different tissues. Similar amounts of RNA at 3 different concentrations were "slot blotted" to a filter. ^{32}P -labelled DNA of *coxII* from maize was hybridized to the filter and autoradiographed.

Gene expression in the mitochondria: protein synthesis

In order to detect differences in gene expression at the level of translation, between fertile and CMS mitochondria, we have analyzed protein synthesis in organello in fertile (normal) and CMS mitochondria. Mitochondria were prepared from root tissue as described by Leaver et al. [20] with minor modifications. Filtered homogenate was centrifuged twice for 10 min. at 3000xg. The supernatant was then centrifuged for 25 min. at 14000xg. The mitochondrial pellet was resuspended in a small volume and layered onto sucrose gradient for further purification. After centrifugation for 45 min. at 17,500rpm in the SW-28 rotor, the mitochondria that accumulated as a band at a density of 1.25-1.35 M sucrose, was recovered and gently diluted to a final concentration of 0.4M sucrose. The diluted mitochondria were centrifuged at 10,000xg for 15 min. Protein synthesis in the isolated mitochondria was carried out as described by Leaver et al. [20] in the presence of ^{35}S -methionine.

To check the quality of our mitochondrial preparation, two types of controls were carried out: 1. Comparison of protein synthesis patterns in the plastid-enriched (second pellet in the organelle purification protocol) to that of purified mitochondria and 2. use of organelle-specific inhibitors in an attempt to specifically inhibit either plastid or mitochondrial protein synthesis (Fig. 12). Comparison of lane 1,2,4,6,7 in Fig. 12, shows clearly the differences in protein synthesized by the plastid enriched pellet and by purified mitochondria. In the presence of erythromycin (100 μM), which is a

potent inhibitor of plastid translation, there was no protein synthesis in the plastid enriched pellet (lane 3) neither was there any effect on the mitochondrial translation product (lane 4). Chloramphenicol (4mM) completely inhibited mitochondrial protein synthesis (lane 5). Cycloheximide (100 μ M), an inhibitor of protein synthesis on cytosolic ribosomes, did not inhibit the mitochondrial protein synthesis (lane 7) but there were some minor differences in the protein pattern. Lack of energy source did not affect the protein synthesis in the plastid enriched pellet in the dark, as expected, but also did not affect the synthesis of protein in the mitochondrial fraction (lane 2 lane 6). In order to exclude bacterial contamination we checked the mitochondrial fraction in EM (data not shown). No bacteria have been observed. In addition, presence of antibiotics in the protein synthesis reaction mixture did not affect its pattern.

A comparison of in organello protein synthesis in sterile and fertile lines (Fig. 13), revealed few minor differences in the polypeptides synthesised in the mitochondria of these genotypes.

Fig. 12: Polypeptides synthesized by isolated mitochondria of BMC-CMS showing controls of plastid protein synthesis and the effects of inhibitors on mitochondrial protein synthesis. Lanes 1-3: Proteins synthesized by the plastid-enriched fraction. Lane 1 - In the presence of "energy mix a" (10mM sodium succinate, 2 mM ADP-ATP); Lane 2 - Without "energy mix a" or GTP; Lane 3 - In the presence of "energy mix a" and 1.0 mM erythromycin. Lanes 4-7: Proteins synthesized by the mitochondrial fraction. Lane 4 - In the presence of "energy mix a" and 1 mM erythromycin; Lane 5 - In the presence of "energy mix a" and 4.0 mM chloramphenicol; Lane 6 - Without "energy mix a" or GTP; Lane 7 - In the presence of "energy mix a" and 1.0 mM cycloheximide.

Fig. 13: Polypeptides synthesized by isolated fertile and sterile sugar beet mitochondria. Mitochondrial translation products were labeled by incubating the isolated mitochondria for 90 min. in a medium containing ^{35}S methionine. The proteins were solubilized in SDS and separated by SDS-PAGE. Labeled polypeptides were detected by autoradiography of the dried gel. Lane 1- Protein synthesized by mitochondria from the "maintainer" fertile line- N xxxz, in the presence of 10mM sodium succinate and 2 mM ADP-ATP (energy mix a); Lane 2- protein synthesized by mitochondria from sterile line, Sxxxz (S-CMS without restorer genes), in the presence of "energy mix a"; Lane 3- protein synthesized by mitochondria from Nxxxz in the presence of 8 mM creatine phosphate, 25 μM creatine phosphokinase 6 mM ATP (energy mix b); Lane 4- protein synthesized by mitochondria from Sxxxz in the presence of "energy mix b"; Lane 5- protein synthesized by mitochondria from Nxxxz in the presence of 20 mM sodium acetate (energy mix c); Lane 6- protein synthesized by mitochondria from Sxxxz in the presence of energy mix c.

Molecular analysis of coxII gene

The gene coxII was found to be located very close to a DNA rearrangement that was found in S-CMS as compared to N. In order to study its primary structure we have cloned from fertile (N) and BMC-CMS, the two EcoRI fragments of the sizes 1.5 kb and 1.95 kb, and from S-CMS the 1.95 kb and 7 kb fragments, that were homologous to coxII from maize(Fig.3).

DNA sequence analysis of the 1.5 kb EcoRI fragment, containing the 5' part of coxII was carried out in order to study the primary structure of the gene. It appears that coxII in sugar beet is very homologous to the gene of maize [24], and that it contains an intron in the same position. the sequence of the first exon of coxII in B. vulgaris (N mitochondria) and almost the entire intron are shown in Fig. 14. Comparison between the deduced amino acid sequence of the first exon in sugar beet (N) and other plants is given in Fig. 15. No differences were found in the sequence between N and BMC-CMS. The S-CMS sequence has not been completed by the time of preparing this report.

The 1.95 kb DNA fragment from all three genotypes was sequenced. It contains a region of 255bp which is homologous to a part of the first exon of coxII (Fig. 16). It is not known whether this DNA is transcribed. In any case, since no differences were found between the three genotypes, we cannot conclude that it is connected to CMS.

Fig. 14: Nucleotide sequence of the 1.5kb fragment from N mitochondria, containing the coxII gene. The deduced amino acid sequence of the first exon is indicated.

10	20	30	40	50	60
*	*	*	*	*	*
G AAT TCA CTG CCC TTG AAC ATG GCG TCT TCC TCT TCT TCA CCG GTA GCG CTT TCA AAG					
70	80	90	100	110	120
*	*	*	*	*	*
AGG GGG AGG CCG CGC AAC AGC GGA GCC AAA AAT CCC GTT TTT TGC TCA GTT GAT GGG TGT					
130	140	150	160	170	180
*	*	*	*	*	*
GCT TCT GAC CTG AAT CAA TGC CGG GAA TAC CAT CGC CGC CAT AAG GTG TGT GAG AGG CAC					
190	200	210	220	230	240
*	*	*	*	*	*
TCT AAG ACT CCT GTC GTT CTT GTT GGA GGG AAG AAG CCG CAG TTT TGC CAA CAC TGT AGC					
250	260	270	280	290	300
*	*	*	*	*	*
GGG TGC AAA AAA ATC ATA AGA GAA GAA AGC AAT GCC CAA AGA CTC CCA TGC CTT TCT TGG					
310	320	330	340	350	360
*	*	*	*	*	*
TTG GAA AAC CCA ACC GGT GAT TTC TGA CAA GTC TTT CTT CAT TTC TCG AGA GAG CGG AGC					
370	380	390	400	410	420
*	*	*	*	*	*
AGT CAA AGA ATG AAC CAA ATG ATT GTT CGA GAA TGG CTA TTC TTC ACA ATG GCT CCT TGT					
Met Ile Val Arg Glu Trp Leu Phe Phe Thr Met Ala Pro Cys					
430	440	450	460	470	480
*	*	*	*	*	*
GAT GCA GCG GAA CCA TGG CAA TTA GGA TTT CAA GAC GCA GCA ACA CCT ATG ATG CAA GGA					
Asp Ala Ala Glu Pro Trp Gln Leu Gly Phe Gln Asp Ala Ala Thr Pro Met Met Gln Gly					
490	500	510	520	530	540
*	*	*	*	*	*
ATA ATC GAC TTA CAT CAT GAT ATC TTT TTC TTC CTC ATT CTT ATT TTG GTT TTC GTA TCA					
Ile Ile Asp Leu His His Asp Ile Phe Phe Phe Leu Ile Leu Ile Leu Val Phe Val Ser					
550	560	570	580	590	600
*	*	*	*	*	*
TGG ATC TTG GTT CGC GCT TTA TGG CAT TTC CAC TAT AAA AAA AAT CCA ATC CCG CAA AGG					
Trp Ile Leu Val Arg Ala Leu Trp His Phe His Tyr Lys Lys Asn Pro Ile Pro Gln Arg					
610	620	630	640	650	660
*	*	*	*	*	*
ATT GTT CAT GGA ACT ACT ATC GAG ATT ATT CGG ACC ATC TTT CCC AGT ATC ATC CTG ATG					
Ile Val His Gly Thr Thr Ile Glu Ile Ile Arg Thr Ile Phe Pro Ser Ile Ile Leu Met					
670	680	690	700	710	720
*	*	*	*	*	*
TTC ATT GCT ATA CCA TCA TTT GCT CTG TTA TAC TCA ATG GAC GAG GTA GTA GTA GAT CCA					
Phe Ile Ala Ile Pro Ser Phe Ala Leu Leu Tyr Ser Met Asp Glu Val Val Val Asp Pro					

730	740	750	760	770	780
* GCC	* ATT	* ACT	* ATC	* AAA	* GCT
Ala	Ile	Thr	Ile	Lys	Ala
790	800	810	820	830	840
* GAT	* TTA	* AGT	* GCA	* ACG	* AAA
TTC	CCG	TCG	TCG	AGA	GAC
850	860	870	880	890	900
* GGT	* AAT	* CTC	* CCA	* TTC	* CCG
910	920	930	940	950	960
* AGT	* TGA	* TTG	* AGG	* TGG	* TTA
970	980	990	1000	1010	1020
* CCA	* TTC	* TTG	* TTG	* CTG	* GAG
1030	1040	1050	1060	1070	1080
* AAT	* AGA	* TGC	* TCA	* GCC	* TGC
1090	1100	1110	1120	1130	1140
* ATA	* GCA	* ATG	* GCA	* TAC	* AAC
1150	1160	1170	1180	1190	1200
* ATC	* CCC	* GCT	* GGC	* AAC	* AAC
1210	1220	1230	1240	1250	1260
* AAC	* CTT	* ATT	* GCC	* TGC	* TCC
1270	1280	1290	1300	1310	1320
* CAG	* GCA	* CCA	* AGC	* ACC	* TTG
1330	1340	1350	1360	1370	1380
* AGC	* CGC	* TCG	* CAG	* TGA	* TGA
1390	1400	1410	1420	1430	1440
* ACC	* TAT	* TCA	* TAG	* AGT	* GAT
1450	1460	1470	1480	1490	1500
* ATA	* GTT	* AGG	* TTA	* AAA	* AAG
GAC	TCA	ACT	TCT	CAG	CTA
GAG	TTG	GGG	ATG	GGA	CCT

						10																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
--	--	--	--	--	--	----	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

Fig. 15: Comparison of the amino acid sequence of the first exon of coxII in sugar beet and in other species. Only the differences are indicated.

```

-50      10      -30      -20      -10      1
*      *      *      *      *      *
TGA CAA GTC TTT CTT CAT TTC TCG AGA GAG CGG AGC AGT CAA AGA ATG AAC CAA ATG ATT
H I

10      20      30      40      50      60
*      *      *      *      *      *
GTT CGA GAA TGG CTA TTC TTC ACA ATG GCT CCT TGT GAT GCA GCG GAA CCA TGG CAA TTA
V R E W L F F T H A P C D A A E P W Q L

AAT TCG AGA ATG GCT ATC TTC ACA ATG GCT CTT TGT GAC GCA GCG GAA CCA TGG CAA TTA
N S R M A I F T H A L C D A A E P W Q L

70      80      90      100      110      120
*      *      *      *      *      *
GGA TTT CAA GAC GCA GCA ACA CCT ATG ATG CAA GGA ATA ATC GAC TTA CAT CAT GAT ATC
G F Q D A A T P H H Q G I I D L H H D I

GGA TTT CAA GAC GCA GCA ACG CCT ATG ATG CAA GGA ATA ATC GAC TTA CAT CAT GAT ATC
G F Q D A A T P H H Q G I I D L H H D I

130      140      150      160      170      180
*      *      *      *      *      *
TTT TTC TTC CTC ATT CTT ATT TTG GTT TTC GTA TCA TGG ATC TTG GTT CGC GCT TTA TGG
F F F L I L I L V F V S W I L V R A L W

TTT TTC TTC CTC ATT CTT ATT TTG GTT TTC GTA TCA TGG ATC TTG GTT CGC GCT TTA TGG
F F F L I L I L V F V S W I L V R A L W

190      200      210      220      230      240
*      *      *      *      *      *
CAT TTC CAC TAT AAA AAA AAT CCA ATC CCG CAA AGG ATT GTT CAT GGA ACT ACT ATC GAG
H F H Y K K N P I P Q R I V H G T T I E

CAT TTC CAC TAT AAA AAA AAT CCA ATC CCG CAA AGG ATT GTT CAT GGA ACT ACT ATC GAG
H F H Y K K N P I P Q R I V H G T T I E

250      260      270      280      290      300
*      *      *      *      *      *
ATT ATT CCG ACC ATC TTT CCC AGT ATC ATC CTG ATG TTC ATT GCT ATA CCA TCA TTT GCT
I I R T I F P S I I L H F I A I P S F A

ATT ATT CCG ACC ATC TTT CCC AGT ATC ATT CTG ATT ACA CAA CTA ATA TTC TTG CTT TCG
I I R A I F P S I I L I T Q L I F L L S

310      320      330      340      350      360
*      *      *      *      *      *
CTG TTA TAC TCA ATG GAC GAG GTA GTA GAT CCA GCC ATT ACT ATC AAA GCT ATT GGA
L L Y S H D E V V V D P A I T I K A I G

GTA GGG GTG GAC CCC ATC CTT GTT GCC CCT TGC AGT GGA TCC GGG TCC TTC CCG GCC TTA
V G V D P I L V A P C S G S G S F R A L

370      380      390      400      410      420
*      *      *      *      *      *
CAT CAA TGG TAT CCG AGT GCG CCT CTT CAG GAA GGT GAT TTA AGT GCA ACG AAA TGC CTT
H Q W Y R S A P L H E G D L S A T K C L

TCT ACC CCG ACA GAT AGG TGC CCA AGC TAG TGG GGC TTC CAC TAC GGA AGA AGA TCT CTT
S T R T D R C P S -

430      440      450      460      470      480
*      *      *      *      *      *
AAA GTG GAA TAT AGT TCG CAA AGC ATC TGG CTT ACT GGT AAT CTC CCA TTC CCG TCG TCG
K V E Y S S Q S I W L T G N L P F P S S

GGA GCA GTT TGT CAT CCA GGA AGA GGA ACT TGC AGA GGG TAG GGT CCG AGG TAT GCC CGA

```

Fig. 16: Comparison of the nucleotide sequence and deduced amino acid sequence of the region of homology between the 1.95kb fragment and *coxII* gene in the N mitochondria.

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 [16]. Sugar beet is considered vulnerable to pathogens and stress because of the uniformity and narrowness of its genetic background [19,27]. All the hybrid varieties currently in agricultural use, carry the same cytoplasm S-CMS, which has been found by Owen [2].

In our research, 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 of 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 different hybridization patterns 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 appear 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: Si-2, Si-3 and Si-4, which have been derived from the N-type, are shown here to contain a 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. The latter possibility is probably the correct one.

In two well-studied cases of CMS in maize [16] and in Petunia [17], it has been shown that CMS is strongly correlated with specific mitochondrial genome rearrangements that involve specific genes. In sugar beet we have found that coxII and atp6 are involved in mtDNA rearrangements between N and S-CMS. In an attempt to understand the molecular basis of CMS in sugar beet, we have looked for differences in expression of these genes between S-CMS and N.

The in organello protein synthesis has indicated differences in the pattern of proteins that were synthesized in the mitochondria of fertile and S-CMS. These differences were too small to establish whether they are qualitative, namely proteins that are synthesized only in S-CMS, or just quantitative ones. In the cases of CMS-T in maize and in Petunia, a major polypeptide was found to be synthesized in the

CMS type only. It has been shown that these proteins were the translation products of a chimeric gene which does not exist in the normal (fertile) genotype and does not have a normal function in the mitochondria. It is possible that in our case too, the polypeptides that were found in the S-CMS are connected to the CMS phenotype, however, since several of them were found, and with the same quantities, it is very likely that their synthesis is enhanced (or perhaps even regulated) by another, yet unidentified gene. Because of this reason and the fact that the differences were very minor ones, we did not follow this line to look for the genes coding for these proteins.

Analysing gene expression at the transcriptional level has revealed major differences between N and S-CMS in the sizes of transcripts of the genes coxII and atp6. Two transcripts of coxII were found in both N and S-CMS (the Japanese genotypes were identical to the S-CMS). One of them was similar in size in the two genotypes but the second one was different. In addition, a small increase in the relative amount of steady state mRNA for coxII, was found in mitochondria of S-CMS, as compared to N, while transcription of other genes was similar. This difference could be related to CMS if, for example, the rate of transcription of one of the mRNA species in the S-CMS is increased relative to the N type.

The transcriptional analysis and the reorganization of the mtDNA in S-CMS relative to the N type, pointed to the possible involvement of coxII in molecular events that are unique to S-CMS. At the same

time, BMC-CMS was found to be similar to the N. For these reasons we have decided to analyze coxII at the molecular level. The existence of what seems to be a pseudogene for coxII, hampered for a long time all our attempts to identify and clone the entire coxII gene. However, when this gene was sequenced it was found that this chimeric coxII has a similar structure and sequence in N, S-CMS and BMC-CMS, and therefore it cannot be concluded at the moment if this "gene" is related to CMS. Further detailed studies of its transcription and also sequencing of its upstream region, are required to clarify this point.

REFERENCES

1. Owen FV (1942) J. Agric. Res. 64: 679-698.
2. Owen FV (1945) J Agric Res 71: 423-440
3. Owen FV (1950) Proc Am Soc sugar beet Technol 6: 191-194
4. Lonsdale D (1987) Plant Physiol Biochem 25: 256-271
5. Fluhr R, Aviv D, Edelman M, Galun E (1983) Theor Appl Genet 65: 289-294
6. Clark EM, Izhar S, Hanson MR (1985) Theor Appl Genet 199:440-445
7. Powling A (1982) Heredity 49: 117-120
8. Powling A (1981) Mol Gen Genet 183: 82-84
9. Powling A, Ellis THN (1983) Theor Appl Genet 65: 323-328
10. Mikami T, Harada T, Kinoshita T (1986) Curr Genet 10: 695-700
11. Hallden C, Bryngelsson T, Bosemark NO (1988) Theor Appl Genet 75:561-568.

12. Mikami T, Kishima Y, Sugiura M, Kinoshita T (1985) Theor Appl Genet 71: 166-171
13. Kishima Y, Mikami T, Hirari A, Sugiura M, Kinoshita T (1987) Theor Appl Genet 73:330-336.
14. Hansen BM, and Marcker KA (1984) Nucl Acid Res 12: 4747-4756
15. Thomas CM (1986) Nucleic Acid Res 14:9353-9370
16. Dewey RE, Siedow JN, Timothy DH, Levings III CN (1988) Science 239:293-295.
17. Young EG, Hanson MR (1987) Cell 50:41-49
18. Kinoshita T (1977) Jap J Breed 27: 19-27
19. Coe GE, Stewart D (1977) J Am Soc Sugar Beet Technol 19: 257-261
20. Leaver CJ, Hack E, Forde BG (1983) Methods Enzymol 97:476-484
21. Sparks RB Dale RMK (1980) Mol Gen Genet 180: 351-355
22. Maniatis T, Fritsch EF, Sambrook J. (1982) Molecular cloning - a laboratory manual. Cold Spring Harbor Laboratory, New York.
23. Isaac PG, Jone VP, Leaver CJ (1985). EMBO J 4: 1617-1623
24. Fox TD, Leaver CJ (1981) Cell 26: 315-323
25. Dawson AJ, Jones VP, Leaver CJ (1984) EMBO J 3: 2107-2113
26. Hooker AL, Smith DR, Lim SM, Beckett JB (1970) Plant Dis Rep 54:708-712
27. Bosemark NO (1979) in: Zeven AC, Harten AM van (1987) Proc Conf Broadening Genet Base Crops. Pudoc, Wageningen, pp 29-35.
28. Dewey RE, Schuster AM, Levings CS and Timothy DH (1985), Proc. Natl. Acad. Sci. USA 82: 1015-1019
29. Dewey RE, Levings CS and Timothy DH (1985) Plant Physiol. 79:914-919

30. Bonen L, Boer PH and Gray MW (1984) EMBO J. 3:2531-2536

DESCRIPTION OF COOPERATION

The three parties of this program have contributed to to the research in the following way: All the different genotypes of sugar beet have been constructed and examined by Dr. Theuerer from the USDA ARS at East Lansing. He also has studied the BMC-CMS and supplied most of the plants for the molecular studies. Some of the plant material was grown in Israel from seed that were sent from Est Lansing. Extraction of nucleic acids from mitochondria of the various genotypes of sugar beet was done mostly in the laboratory of Dr. McIntosh but also in Jerusalem. RNA analyses was carried out mostly in Dr. McIntosh's laboratory, but the coxII RNA was done also in Israel. Most of the molecular work including: Southern analyses of mt DNA, cloning and sequencing the coxII genes and the in organello protein synthesis, were done in jerusalem.

Some experiments, mainly those that required fresh plant material, were carried out during two short visits to East Lansing of the Israeli PI. Discussios with all the PIs during these visits helped in coordinating the research.

EVALUATION OF THE RESEARCH ACHIEVEMENTS

The most important achievement of this research concerns the BMC-CMS genotype. This research has established, at the molecular level, that BMC-CMS is a novel genotype for cytoplasmic male sterility in B. vulgaris L. As already discussed in the body of the report, there is a great need for a new CMS type in sugar beet, to be an alternative for the extensively used S-CMS. The molecular evidence for the uniqueness of BMC-CMS makes this genotype very attractive for use in future breeding of sugar beet.

It was shown here that the other CMS genotypes that have been reported by Japanese breeders, contain a mitochondrial genome that is identical to S-CMS and therefore of no particular importance for future breeding.

As to the molecular phenomena that were found in the CMS genotype, these were not shown to be directly connected to CMS and, like in other reported cases of CMS, are assumed to correlate to a specific mtDNA rather than to have a direct causal relationship to CMS. The finding of a coxII-homologous sequence in the mitochondrial genome and the small increase of coxII mRNA in S-CMS could be connected to CMS and this possibility should be further studied.

The structure of the gene coxII in sugar beet was found to be conserved with other plants. This finding, together with the discovery of a portion of coxII in another location in the mitochondrial genome, shed light on the structure and expression of mitochondrial genes in plants.

PUBLICATIONS

Mann V, McIntosh L, Theurer C and Hirschberg J (1989)

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

Theor. Appl. Genet. (in press).

Two additional manuscripts: on the transcription of mitochondrial genes in sugar beet and on the sequence analysis of coxII in sugar beet, will be submitted for publication during 1989.