Theor Appl Genet (1985) 71:166–171

Organelle genome diversity in sugar beet with normal and different sources of male sterile cytoplasms

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Received April 22, 1985; Accepted May 15, 1985
Communicated by K. Tsunewaki

Summary. Mitochondrial (mt) and chloroplast (ct) DNAs from sugar beet lines carrying normal and introduced sources of male sterile cytoplasms have been characterized and compared on the basis of restriction enzyme analysis. Normal cytoplasm was shown to contain mt and ctDNAs which differed from those of the male sterile cytoplasms examined in the present investigation. On the other hand, four groups of male sterile cytoplasms could be differentiated by their own characteristic mtDNA digest patterns, while two were separated by ctDNA comparisons. In addition, a greater degree of variability of the mitochondrial genome is suggested. Our results also imply strict maternal inheritance of mt and ctDNAs. Thus, the organelle DNA assay provides a positive and alternative means of identifying various male sterile cytoplasms.

Key words: Sugar beet – Cytoplasmic male sterility – Mitochondrial and chloroplast DNAs – Restriction endonuclease fragment analysis

Introduction

Cytoplasmic male sterility (CMS), a maternally inherited trait, has been very rigourously studied because it has provided an efficient way of producing commercial hybrid seeds by preventing self-fertilization of the seed parent. Most of the sugar beet hybrid varieties in Japan have been produced with the aid of a single source of CMS, which was discovered by Owen (1945). Restriction endonuclease fragment analysis has revealed characteristic differences between mitochondrial (mt) DNA from normal (N) and male sterile (S) cytoplasms of sugar beet, whereas apparently less variation exists in chloroplast (ct) DNA from comparable cytoplasms (Powling 1982; Powling and Ellis 1983; Mikami et al. 1984a, b). It has been also found that N and S cytoplasms possess different small circular mtDNA species or combinations of species (Powling 1981; Hansen and Marcker 1984). These results indicate mtDNA to be a carrier of determinants responsible for the sterility trait in sugar beet, although the contribution of ctDNA cannot be entirely excluded.

After the initial discovery of CMS by Owen, the search for, and the artificial induction of, new and different forms of CMS have been conducted in two laboratories (Oldemeyer 1957; Kinoshita 1976, 1977; Kinoshita et al. 1982). Oldemeyer extracted distinct sources of CMS from a wild beet collection of the USDA and developed a series of male sterile lines which are near-isogenic except for the constitution of cytoplasmic genes. The purpose of the present investigation was to survey the cytoplasmic genome variation found in Oldemeyer's CMS collection by examining mt and ctDNAs using restriction enzyme analysis.

Materials and methods

Plant materials

Sugar beet lines used in this study are listed in Table 1. The CMS lines of I-12CMS (R) through I-12CMS (8) possess different sources of cytoplasm but the nuclear genotype equivalent to that of a sugar beet maintainer (Type-0) line, I-12-61L. These materials were provided by the courtesy of Dr. R. K. Oldemeyer, the Great Western Sugar Co. TK81-MS and TK81-0 are CMS and its maintainer inbred lines which were bred in the Hokkaido National Agricultural Experiment Station.

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Preparation of mt and ctDNAs

Taproot and well-expanded leaves of greenhouse grown plants were used to prepare mt and ctDNAs, respectively. Intact mitochondria and chloroplasts were isolated as previously described (Mikami et al. 1984a). Final pellets of either organelle were lysed with 2% Sarkosyl and digested with proteinase K (100 μg/ml) at 37 °C for 2 h. The DNA was purified by two phenol-chloroform extractions and ethanol precipitation followed by preparative CsCl-ethidium bromide centrifugation. After removal of ethidium bromide with isopropyl alcohol, the DNA was dialyzed against 10 mM Tris-HCl buffer (pH 7.8) containing 10 mM KCl and 1 mM EDTA and then ethanol precipitated as described (Sugiura and Kusuda 1979).

Restriction enzyme analysis and Southern hybridization

About 1.5 μg of DNA was digested with BamHI, EcoRI, HindIII or SmaI (Takara Shuzo Co. Ltd) as directed by the manufacturer. The restriction fragments were separated by electrophoresis in 0.4 or 0.7% agarose horizontal slab gels run at 1 mA/cm for 20 h. Gel staining, ultraviolet fluorescence photography and Southern blot hybridization were performed essentially as described by Sugiura and Kusuda (1979). A 11.5×10^6 d BamHI fragment of tobacco ctDNA which contains most of the small single-copy region was used as probe for hybridization experiment. Molecular weight markers were HindIII and EcoRI single or double digest fragments of phage λDNA.

Results

Comparison of mtDNAs

Male sterile lines of I-12CMS (R) through I-12CMS (8) carry the different sources of cytoplasms and were backcrossed to a uniform maintainer line, I-12-61L, for at least six generations. Oldemeyer and his co-workers recognized the differential fertility restoration reactions of these cytoplasms with six inbred genotypes, indicating the genetic variation in male sterility inducing cytoplasms from distinct origins (Suzuki et al. 1974). In the present study, restriction enzyme analysis of mt and ctDNAs provided evidence for molecular heterogeneity of organelle genomes among male sterile cytoplasms derived from cultivated and wild beets.

Figure 1 shows the electrophoregrams of HindIII and SmaI restriction fragments obtained with the mtDNA prepared from normal and CMS lines. In the case of SmaI digestion, N (TK81-0 and I-12-61L) and the widely used S (TK81-MS and I-12CMS (R)) cytoplasms were characterized by at least 40 and 38 fragments of molecular weights greater than 0.7×10^6 d, respectively. As shown in Table 1, N and S mtDNAs

Table 1. Restriction enzyme analysis of mt and ctDNAs from normal and different sources of male sterile cytoplasms

Strain	Fertile / Sterile	Cytoplasmic source	mtDNA						ctDNA							
			EcoRI		HindIII		SmaI		BamHI		EcoRI		HindIII		SmaI	
			T[a]	S[b]	T	S	T	S	T	S	T	S	T	S	T	S
TK81-0	F	Japanese sugar beet variety	45	–	35	–	40	–	22	–	27	–	26	–	15	–
TK81-MS	S	do	41	10	36	14	38	15	22	0	27	0	25	0	15	0
I-12-61L	F	American sugar beet variety	45	0	35	0	40	0	22	0	27	0	26	0	15	0
I-12CMS (R)	S	do	41	10	36	14	38	15	22	0	27	0	25	0	15	0
I-12CMS (2)	S	Wild beet from Turkey	44	6	38	9	34	7	22	0	27	0	27	2	15	0
I-12CMS (3)	S	Wild beet from Pakistan	44	6	38	9	37	11	22	0	27	0	27	2	15	0
I-12CMS (4)	S	Wild beet from Turkey	41	10	36	14	38	15	22	0	27	0	25	0	15	0
I-12CMS (5)	S	Wild beet from Turkey	41	10	36	14	38	15	22	0	27	0	25	0	15	0
I-12CMS (7)	S	Wild beet from Manchuria	44	6	38	9	36	8	22	0	27	0	27	2	15	0
I-12CMS (8)	S	Wild beet from Turkey	41	10	36	14	38	15	22	0	27	0	25	0	15	0

[a] Total fragments
[b] Cytoplasm-specific fragments which are not common with TK81-0

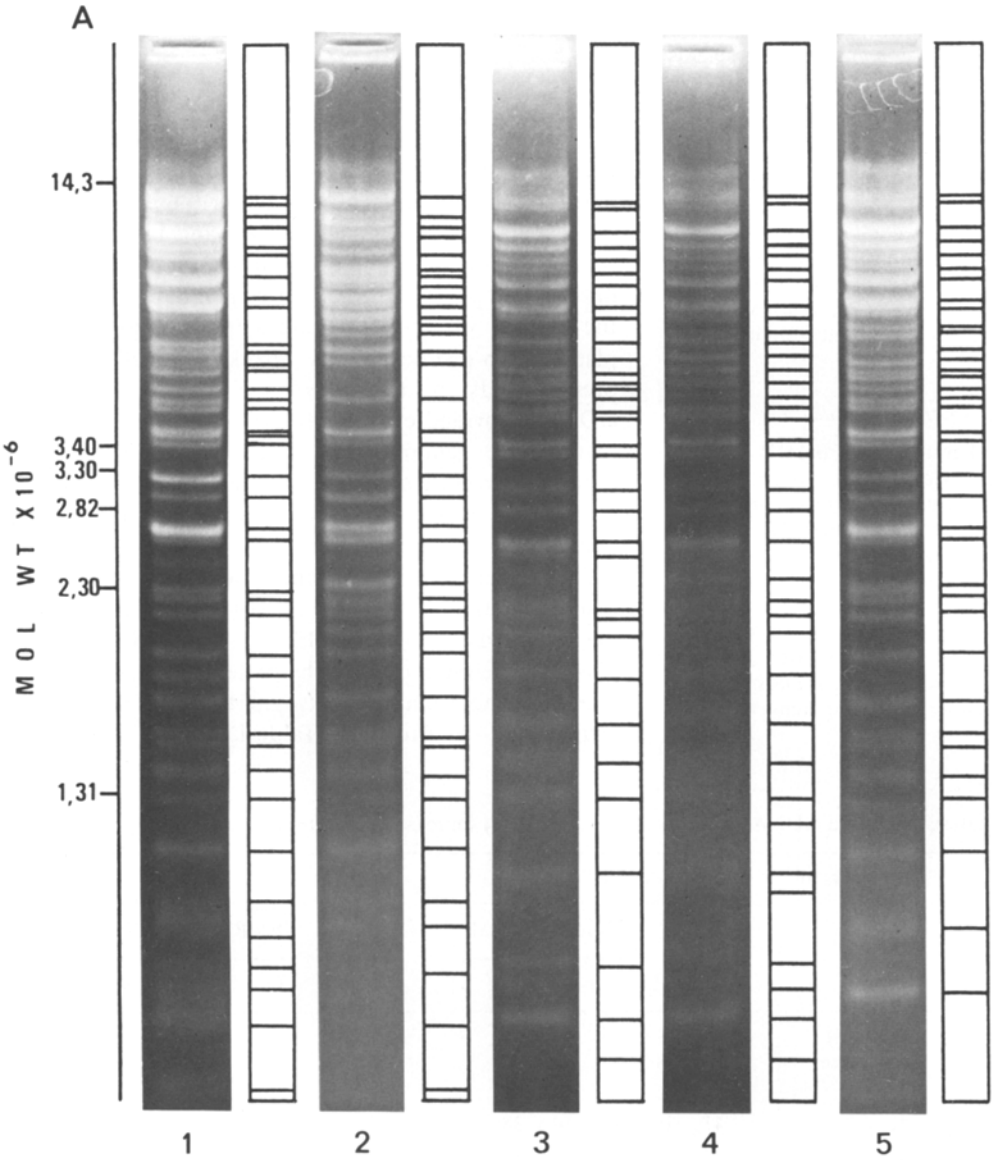

Fig. 1. *SmaI* (**A**) and *HindIII* (**B**) restriction fragment patterns of mtDNAs. The DNA fragments were separated by electrophoresis in 0.4% (*SmaI*) or 0.7% (*HindIII*) agarose slab gels. *Lanes 1* and *6*: I-12-61L; *Lanes 2* and *7*: I-12CMS (R); *Lanes 3* and *8*: I-12CMS (2); *Lane 4*: I-12CMS (3); *Lane 5*: I-12CMS (7)

produced 17 and 15 of cytoplasm-specific bands, respectively, and had 23 in common.

The SmaI analysis revealed four groups of mtDNAs from alternative CMS sources; members of the first group (I-12CMS (4), I-12CMS (5) and I-12CMS (8)) displayed restriction profiles indistinguishable from that characteristic of S cytoplasm; the other groups, whose cleavage patterns could be differentiated from that of N or S mtDNA, consisted of I-12CMS (2), I-12CMS (3) and I-12CMS (7). The SmaI restriction patterns of mtDNA from the latter three cytoplasms were found to differ from one another, and, as a result, the lines were assigned to three separate groups.

Analysis of mtDNA with EcoRI and HindIII separated the seven I-12CMS lines into two groups (Fig. 1, Table 1). However, the same analysis failed to reveal any heterogeneity in the mtDNAs among I-12CMS (2), I-12CMS (3) and I-12CMS (7). The results obtained here are in general agreement with fertility restoration data from field experiments (Suzuki et al. 1974; Kinoshita and Mikami, unpublished).

Comparison of ctDNAs

Chloroplast DNAs from normal and male sterile lines were digested with BamHI, EcoRI, SmaI and HindIII

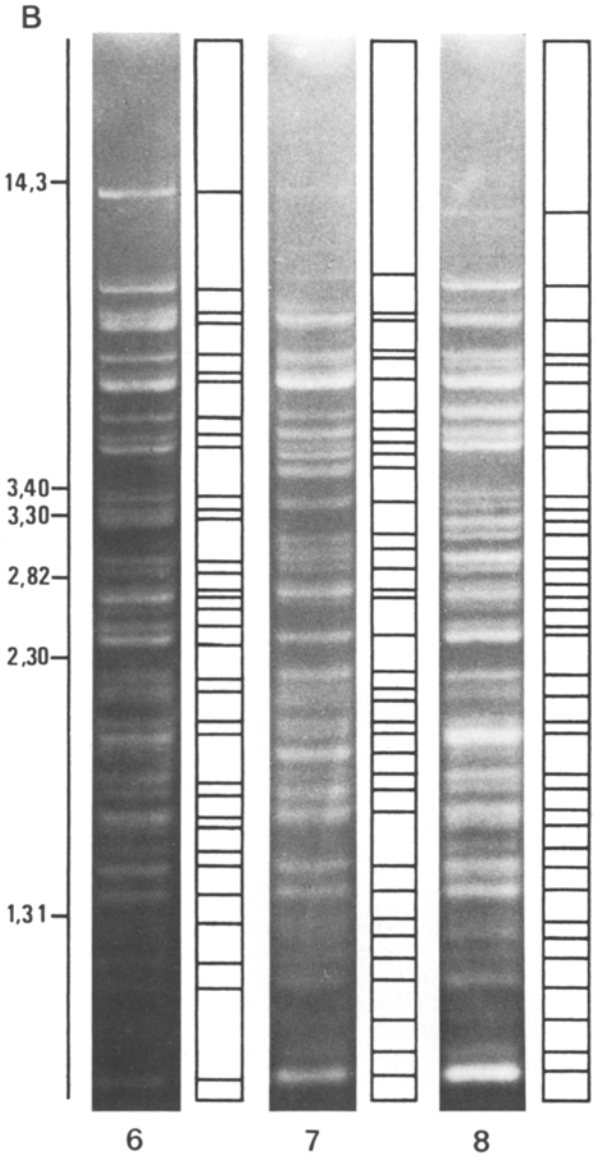

Fig. 1

and the resulting fragments were fractionated by gel electrophoresis. The first three of these enzymes did not distinguish ctDNAs from normal and CMS lines examined (Table 1).

Fragment patterns generated by the fourth enzyme, HindIII, revealed three groups of ctDNAs (Fig. 2). As reported in our previous papers (Mikami et al. 1984a, b), HindIII cleavage of N (TK81-0 and I-12-61L) and S (TK81-MS and I-12CMS (R)) ct DNAs led to 26 and 25 bands, respectively, with 25 common bands. Densitometric tracings and a Southern hybridization experiment demonstrated one missing band (3.5×10^6 d) and replacement of this band by two fragments of 1.95 and 1.7×10^6 d in S ctDNA.

The other six CMS lines were divided into two groups based upon the HindIII analysis. The first group, consisting of I-12CMS (4), I-12CMS (5) and I-12CMS (8), shared ctDNA restriction patterns with S cytoplasm. In contrast the remaining CMS lines were assigned to a separate group by two cytoplasm-specific fragments of 11.5 and 3.0×10^6 d. The combined molecular weight of these two fragments is 14.5×10^6 d, which is equal to the original size of the largest HindIII fragment of N and/or S ctDNA. It is thus suggested that the ctDNAs of CMS mutants of the second group differ from N ctDNA by at least a single HindIII site.

A 3.5×10^6 d HindIII band was previously found to hybridize to a 12.5×10^6 d BamHI fragment of tobacco ctDNA which contains the left margin of the large single-copy region (Mikami et al. 1984b). The largest HindIII band (14.3×10^6 d), on the other hand, hybridized with a 11.5×10^6 d BamHI ctDNA fragment of tobacco which occupies most of the small single-copy region (Fig. 2). The linear arrangement of common sequence elements is highly conserved among angiosperm chloroplast genomes containing the inverted repeat (Palmer and Thompson 1982; Palmer et al. 1983). Therefore, the differences among beet chloroplast genome organizations are likely to be due to the introduction of HindIII sites in the above-mentioned regions of ctDNA. It is also noteworthy that the percentage of cytoplasm-specific fragments on the given mtDNA digestion profiles is much higher when compared with ctDNA restrictions (Table 1). This indicates that mtDNAs are more diversified than the corresponding ctDNAs.

Discussion

The present paper, together with our previous reports (Mikami et al. 1984a, b), indicates that considerable organelle DNA diversity is apparent among sugar beet lines carrying normal and male sterile cytoplasms from distinct sources. Five groups of mtDNAs were recognized by studying the electrophoretic patterns formed after treatment with restriction endonucleases while three groups of chloroplast genomes were identified on the basis of ctDNA comparisons. Further, we have shown a greater degree of variability of the mitochondrial genomes in comparison with chloroplast genomes. This suggests, but by no means proves, that mtDNA encodes the defect causing the CMS trait in beet.

Molecular heterogeneity associated with CMS has been previously detected in maize and sorghum. In maize, three major types of CMS (designated as CMS-T, CMS-S and CMS-C) have been classified genetically by their differential responses to specific nuclear gene(s) involved in fertility restoration (Beckett 1971; Gracen and Grogan 1974). This

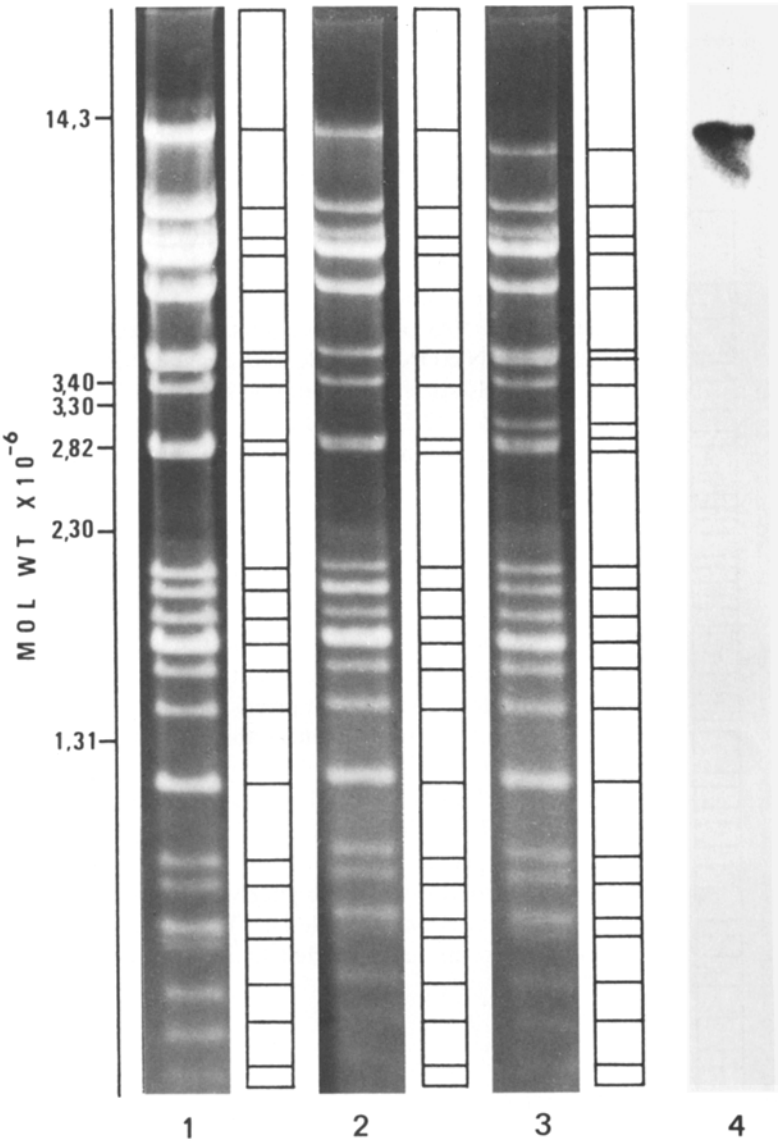

Fig. 2. *HindIII* restriction fragment patterns (*1–3*) of ctDNAs and autoradiograph (*4*) of *HindIII* digests of I-12-61L ctDNA hybridized to ^{32}P-labeled tobacco ctDNA probe. The DNA fragments were separated by electrophoresis in 0.7% agarose slab gel. A tobacco probe contains most of the small single-copy region. *Lane 1:* I-12-61L; *Lane 2:* I-12CMS(R); *Lane 3:* I-12CMS(2)

classification was confirmed by recent work dealing with the restriction enzyme analysis of mtDNA (Pring and Levings 1978) and the comparative study of mitochondrial translation products (Forde and Leaver 1980; Forde et al. 1980). Likewise, sorghum has been reported to contain at least eight different CMS types, depending on restriction digest patterns of mt and ctDNAs (Pring et al. 1982; Conde et al. 1982). Thus, the DNA assay is shown to provide a convenient way of recognizing and characterizing cytoplasmic variations.

Our data also demonstrated the absence of paternal transmission of organelle DNA. As mentioned in a foregoing section, a series of I-12CMS lines were backcrossed to I-12-61L for more than six generations. Each generation then represents an additional opportunity for paternal (I-12-61L) mt and ctDNAs to be expressed in the resultant progeny. The absence of paternal organelle DNA-specific fragments in the organelle DNAs from I-12CMS lines indicates strict maternal inheritance of mt and ctDNAs in our material.

In order to extend the exploitation of CMS, there is a need to understand the biochemical and molecular mechanism(s) underlying the trait. Kinoshita (1976, 1977) and Kinoshita et al. (1982) succeeded in inducing new forms of male sterile cytoplasms in sugar beet by means of γ-ray irradiation and treatments with some chemicals. More recently, streptomycin has been found to be effective for cytoplasmic reversion to male fertility (Kinoshita et al., submitted). These mutants may be very useful materials for molecular approaches to establish a causal relationship between mutation in mitochondrial genotypes and the phenotypic expression in the whole plant.

Acknowledgements. Seeds were kindly supplied by Drs. R. K. Oldemeyer and A. Suzuki of the Great Western Sugar Co. and Hokkaido National Agricultural Experiment Station. We are grateful to Dr. K. Shinozaki for his helpful suggestions. This work was supported in part by Grants-in-Aids for Scientific Researches (58480030 and 58560001) from the Ministry of Education, Science and Culture, Japan.

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