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Nuclear-mitochondrial interactions in cytoplasmic male-sterile Sorghum

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Summary. Variation in mitochondrial genome organization and expression between male fertile and sterile nuclear-cytoplasmic combinations of sorghum has been examined. Cytoplasmic genotypes were classified into eleven groups on the basis of restriction endonuclease digestion of mitochondrial DNA (mtDNA) and five groups on the basis of mitochondrial translation products. These cytoplasms were further characterized by hybridization of specific gene probes to Southern blots of *Eco*RI digested mtDNA, and identification of the fragment location of four mitochondrial genes. Variation was observed in the genomic location and copy number of the F_1 ATPase α-subunit gene, as well as the genomic location and gene product of the cytochrome *c* oxidase subunit I gene. The effect of nuclear genotype on mitochondrial genome organization, expression and the presence of two linear plasmid-like mtDNA molecules was examined. Our results indicate that nuclear-mitochondrial interactions are required for regulation of mitochondrial gene expression. When a cytoplasm is transferred from its natural to a foreign nuclear background some changes in the products of in organello mitochondrial protein synthesis occur. In a number of cytoplasmic genotypes these changes correlate with the expression of cytoplasmic male sterile phenotype, suggesting a possible molecular basis for this mutation.

Key words: Cytoplasmic male sterility – Sorghum – Mitochondrial gene expression – Nuclear-cytoplasmic interactions

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Introduction

Cytoplasmic male sterility (CMS) is a maternally inherited trait in which pollen development or normal anther dehiscence is impaired but female fertility is normal (Duvick 1965; Edwardson 1970). The CMS phenotype is widely used in the commercial production of F_1 hybrid seed because self-pollination of the female parent is prevented. In sorghum (*Sorghum bicolor*), as in maize (*Zea mays*), CMS is observed when a cytoplasm is transferred to a nuclear background which lacks fertility restoration alleles. Thus, CMS appears to result from nuclear-cytoplasmic incompatibility.

In maize, several lines of evidence suggest that CMS originated as mutations in the mitochondrial genome. Three CMS cytoplasms (T, S and C) have been identified on the basis of specific nuclear alleles which restore pollen fertility (Beckett 1971). In addition, these lines can be distinguished from one another and fertile, normal (N) cytoplasm by differences in organization and expression of the mitochondrial genome. In the CMS T cytoplasm the synthesis of a variant 13,000 dalton (13 KD) mitochondrial translation product is correlated with expression of the CMS phenotype (Forde et al. 1978; Forde and Leaver 1980; Dixon et al. 1982). A characteristic of CMS lines with S cytoplasm is the presence of 2 linear plasmid-like mtDNAs (S plasmids) and the synthesis of additional high molecular weight mitochondrial polypeptides (Forde and Leaver 1980).

Since specific nuclear genes restore pollen fertility, the effect of nuclear genotype on mitochondrial genome organization and expression has been examined. In maize, it has been found that the mtDNA restriction patterns of CMS or nuclear restored lines with the same cytoplasmic genotype are identical (Laughnan and Gabay-Laughnan 1983; Leaver et al., unpublished results). Only slight differences in the stoichiometric amounts of mtDNA restriction fragments are detected when maize cytoplasms are backcrossed into various nuclear backgrounds (Levings and Pring 1977; Borck and Walbot 1982). However, nuclear genotype can have a marked influence on the synthesis of variant mitochondrial polypeptides by CMS lines. For example, when CMS T cytoplasm is back-

crossed into a fertility restoring nuclear background synthesis of the variant 13 KD mitochondrial polypeptide is suppressed (Forde and Leaver 1980). In S cytoplasm, nuclear genotype influences the relative abundance of the individual S plasmids and the synthesis of variant mitochondrial polypeptides associated with these plasmids (Laughnan and Gabay-Laughnan 1983; Escote et al. 1986; Liddell and Leaver, in preparation). Genome mapping studies have revealed that the mitochondrial chromosomes of CMS-S are predominantly linear because of the covalent attachment of a S plasmid at one end (Schardl et al. 1984). In cytoplasmic reverants of S cytoplasm with M825 nuclear background the S plasmids are lost and the mitochondrial chromosomes recircularize (Schardl et al. 1985). However, when S cytoplasm is maintained in WF9 nuclear background the S plasmids are not lost (Escote et al. 1986) and variant high molecular weight mitochondrial polypeptides are still synthesized (Liddell and Leaver, in preparation).

Several independent studies have shown that cytoplasms of sorghum can be distinguished by mtDNA restriction endonuclease digestion patterns, presence of plasmid-like mtDNA molecules and synthesis of variant mitochondrial translation products (Pring et al. 1982; Conde et al. 1982; Dixon and Leaver 1982).

In this paper we report the effect of varying nuclear genotype on mitochondrial genome organization, gene location and expression in a range of sorghum cytoplasms. These analyses and those reported previously are combined to give a comprehensive classification of sorghum cytoplasmic genotypes.

Materials and methods

Sorghum genetic stocks

Seed stocks with specific nuclear-cytoplasmic genotypes and fertility status (Table 2) were supplied by G. Dalton of Pioneer Hi-bred International, Plainview, TX and Dr. K. Schertz, Texas A & M University, College Station, TX. Lines with hybrid nuclear genotypes (eg. Kafir × IS2483C nucleus) are F_1 progeny.

Isolation of mitochondrial DNA, digestion and hybridization to ^{32}P-labelled DNA clones

Mitochondria were isolated from five-day-old etiolated coleoptiles (Leaver et al. 1983). MtDNA was purified as described by Dawson et al. (1984), digested with restriction endonucleases according to the enzyme manufacturers' suggestions. MtDNA was fractionated by electrophoresis in a 0.8% (w/v) agarose gels (SeaKem) using 89 mM Tris, 89 mM boric acid, 2 mM EDTA (pH 8.3), stained with EtBr (0.5 µg/ml) and photographed under UV illumination. Gels were transferred to nitrocellulose (Southern 1975), hybridized to ^{32}P-labelled probes (Rigby et al. 1978; Messing 1983) under stringent conditions (5X SSC, 1X Denhardt's solution, 0.1% (w/v) SDS, and 100 µg/ml carrier DNA at 65 °C), washed twice for 15 min in 2X SSC, 0.1% (w/v) SDS and 0.1X SSC, 0.1% (w/v) SDS and exposed to X-ray film. Maize mitochondrial gene probes were: *COX*I clone M3B2 (Isaac et al. 1985a); *COX*II clone pZmE1 (Fox and Leaver 1981); *COB* clone ZmEH680 (Dawson et al. 1984); *ATP*A clone RISalD5 (Isaac et al. 1985b; PG Isaac, personal communication). The sorghum mitochondrial gene probe was clone pSM4.3, which contains the Milo *COX*I gene and flanking sequences (Bailey-Serres et al. 1986). DNA fragment sizes were estimated by running *Hind*III digested lambda-phage DNA in parallel gel lanes.

In organello protein synthesis

Mitochondria were isolated and allowed to synthesize proteins in the presence of L-[^{35}S]-methionine (Leaver et al. 1983). Mitochondrial proteins were fractionated by electrophoresis in 16% (w/v) SDS-polyacrylamide gels, and labelled translation products were detected by exposure to X-ray film. The molecular weight of labelled polypeptides were estimated by running in parallel the following protein markers of known molecular weight: Bovine Serum Albumin (68,000), Catalase (60,000), Aldolase (40,000) Carbonic Anhydrase (29,000), Trypsin Inhibitor (21,000), Myoglobin (17,200) and Lysozyme (14,300).

Results

MtDNA restriction digestion pattern and restriction fragment location of mitochondrial genes

Three normal (male fertile) cytoplasms, and six CMS cytoplasms in various nuclear backgrounds were used in these studies (Tables 1 and 2). To examine the effect of nuclear genotype on mitochondrial genome organization we characterized the mtDNA of these lines by restriction endonuclease digestion analysis and determination of the restriction fragment location of the genes encoding cytochrome *c* oxidase subunits I (*COX*I) and II (*COX*II), apocytochrome (*COB*), and the α-subunit of F_1 ATPase (*ATP*A). MtDNA was isolated, digested with *Eco*RI and fractionated by agarose gel electrophoresis (Fig. 1 A). Similar patterns were observed for the genotypes: Kafir and Martin; Milo and IS12662C; 9E and IS7920C; M35-1 and IS1112C cytoplasm. A unique *Eco*RI digestion pattern was observed for the normal cytoplasm IS2483C. The fractionated mtDNA was transferred to nitrocellulose and probed with a ^{32}P-labelled clone containing part or an entire maize mitochondrial gene (*COX*I, *COX*II, *COB*, or *ATP*A (see "Methods")) so that the *Eco*RI fragment location of the homologous sorghum mtDNA sequence could be identified.

The maize *COX*I probe (Isaac et al. 1985a) hybridized to one of two *Eco*RI fragments depending upon the cytoplasmic genotype (Fig. 1 B, Table 2). A 4.3 kb *Eco*RI mtDNA fragment was identified in Kafir, Milo, IS12662C, IS1112C and M35-1 cytoplasms. A 10.4 kb *Eco*RI mtDNA fragment hybridized to the *COX*I gene probe in 9E and IS7920C cytoplasms. The observed difference in *Eco*RI fragment location correlates with the synthesis of a larger COI polypeptide by 9E and IS7920C mitochondria (Bailey-Serres et al. 1986).

No difference in the *Eco*RI fragment location of *COX*II (Fox and Leaver 1982) or *COB* (Dawson et al. 1984) was observed amongst these cytoplasms. The maize *COX*II probe hybridized to a 1.6 kb *Eco*RI fragment and to a lesser extent to a 1.5 kb *Eco*RI fragment in all nuclear-cytoplasmic combinations examined

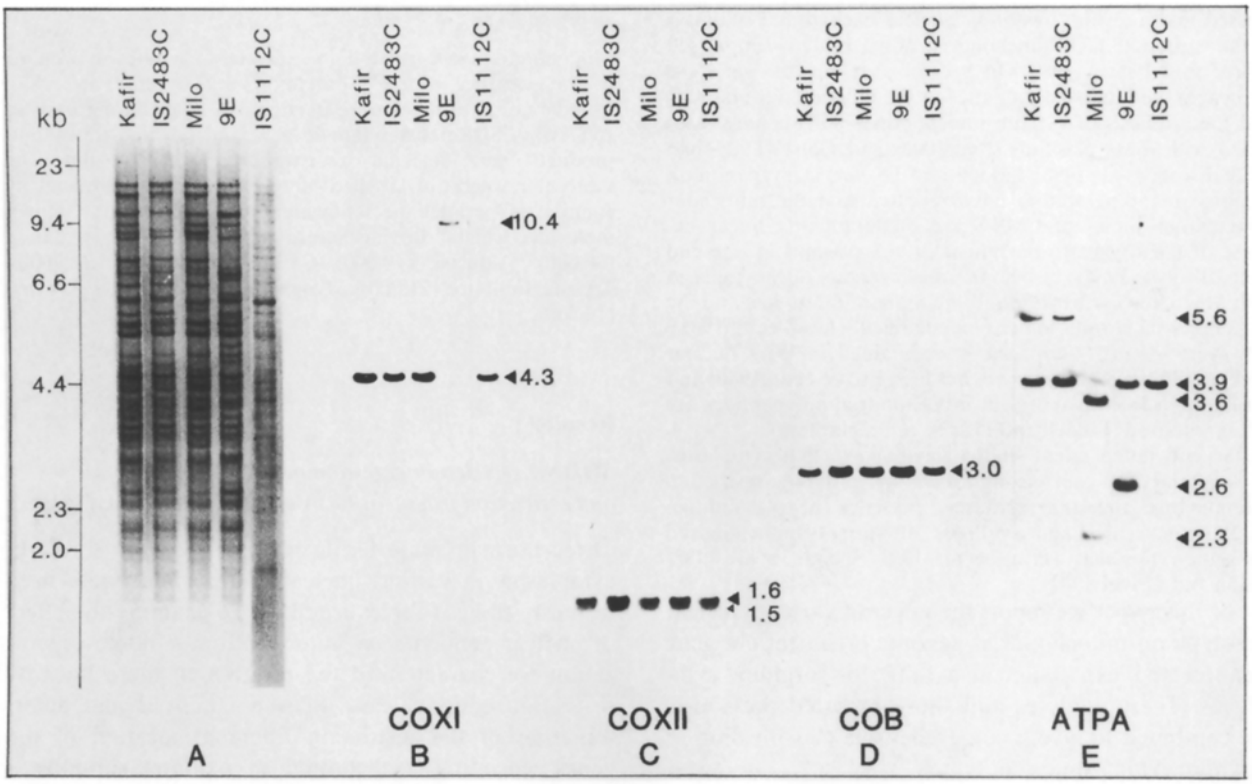

Fig. 1. Identification of *Eco*RI generated MtDNA fragments containing *COX*I, *COX*II, *COB*, and *ATP*A genes of sorghum. MtDNA from IS2483C, Kafir, Milo and 9E cytoplasm was digested with *Eco*RI, fractionated by electrophoresis in a 0.8% (w/v) agarose gel, stained with EtBr and photographed (*A*). The mtDNA was transferred to nitrocellulose and probed sequentially with a ^{32}P-labelled clones containing a portion of a maize mitochondrial gene and autoradiographed. The Southern blot was washed before re-probing. The *Eco*RI fragment(s) (kb) which hybridized to: *COX*I (*B*), *COX*II (*C*), *COB* (*D*), and *ATP*A (*E*). Molecular weight markers were *Hind*III restriction fragments of lambda phage-DNA

Table 1. Taxonomic classification of sorghum lines studied

Line	Cytoplasm designation	Origin	Race	Group
Normal cytoplasms				
Kafir[a]	–	S. Africa	Kafir	Cafforum
Martin[a]	–	S. Africa	Kafir	Cafforum
IS2483C[f]	–	Sudan	Bicolor	Dochna
CMS cytoplasms				
Milo[b]	–	Africa	Durra	Milo-Subglabrenscens
IS12662C[a]	A2	Ethiopia	Caudatum	Zerazera
IS1112C[c]	A3	India	Durra	Durra-Subglabrenscens
IS1054[d]	M35-1	India	Durra	Maldandi
IS17218(9E)[e]	9E	Nigeria	Guinea	Conspicuum
IS7920C[e]	A4	Nigeria	Guinea	Conspicuum

References: [a] Stephens and Holland 1954; [b] Schertz and Ritchey 1978; [c] Quinby 1980; [d] Rao 1962; [e] Webster and Singh 1964; [f] The IS/C number is the International Sorghum designation of individual lines

Table 2. Sorghum lines examined

Nucleus	Cytoplasm	Male fertility status	MtDNA restriction endonuclease digestion pattern	*Eco*RI fragment location of		Plasmid like MtDNAs	Variant polypeptide (KD)
				*COX*I	*ATP*A		
	Normal cytoplasms						
Kafir	Kafir	MF	Kafir	4.3	5.6, 3.9	–	–
Martin	Martin	MF	Kafir	4.3	5.6, 3.9	–	–
IS2483C	IS2483C	MF	IS2483C	4.3	5.6, 3.9	–	–
	CMS cytoplasms						
Milo	Milo	MF	Milo	4.3	3.6, 2.3	–	65 +
Martin	Milo	MS	Milo	4.3	3.6, 2.3	–	65 + + +
Kafir	Milo	MS	Milo	4.3	3.6, 2.3	–	65 + + +
Kafir × IS2483C	Milo	MS	Milo	4.3	3.6, 2.3	–	65 + + +
Kafir × IS12662C	Milo	MS	Milo	4.3	3.6, 2.3	–	65 + + +
Kafir × IS1112C	Milo	MS	Milo	4.3	3.6, 2.3	–	65 + + +
Kafir × M35-1	Milo	MS	Milo	4.3	3.6, 2.3	–	65 + + +
IS12662C	IS12662C	MF	Milo	4.3	3.6, 2.3	–	65 +
Kafir	IS12662C	MS	Milo	4.3	3.6, 2.3	–	65 + + +
Kafir × IS2483C	IS12662C	MS	Milo	4.3	3.6, 2.3	–	65 + + +
IS1112C	IS1112C	MF	IS1112C	4.3	3.9	+	82 + + + 12 +
Kafir	IS1112C	MS	IS1112C	4.3	3.9	+	82 + + + 12 + + +
Yellow Feterita	IS1112C	MS	IS1112C	4.3	3.9	+	82 + + + 12 + + +
Kafir × IS2483C	IS1112C	MS	IS1112C	4.3	3.9	+	N.D.
M35-1	M35-1 (A)	MF	Milo	4.3	3.6, 2.3	–	–
M35-1	M35-1 (B)	MS	M35-1	4.3	3.9	+	82 + + +
Kafir	M35-1 (B)	MS	M35-1	4.3	3.9	+	82 + + +
Yellow Feterita	M35-1	MS	M35-1	4.3	3.9	+	82 + + +
Kafir × IS2483C	M35-1	MS	M35-1	4.3	3.9	+	82 + + +
Kafir × Milo	M35-1	MS	M35-1	4.3	3.9	+	82 + + +
Kafir × IS12662C	M35-1	MS	M35-1	4.3	3.9	+	82 + + +
9E	9E	MF	9E	10.4	3.9, 2.6	–	42 COI
Kafir	9E	MS	9E	10.4	3.9, 2.6	–	42 COI
Martin	9E	MS	9E	10.4	3.9, 2.6	–	42 COI
Kafir × IS2483C	9E	MS	9E	10.4	3.9, 2.6	–	42 COI
Kafir × Kaoliang	9E	PF	9E	10.4	3.9, 2.6	–	42 COI
Kafir Korgi	9E	PF	9E	10.4	3.9, 2.6	–	42 COI
Martin × IS1112C	9E	PF	9E	10.4	3.9, 2.6	–	42 COI
Martin × IS12662C	9E	PF	9E	10.4	3.9, 2.6	–	42 COI
Martin × TX430	9E	PF	9E	10.4	– –	–	42 COI
IS7920C	IS7920C	MF	IS7920C	10.4	3.9, 2.6	–	42 COI
Kafir	IS7920C	MS	IS7920C	10.4	3.9, 2.6	–	42 COI
Kafir × IS2483C	IS7920C	MS	IS7920C	10.4	3.9, 2.6	–	42 COI

MF = male fertile; MS = male sterile; PF = partial fertile; N.D. = not determined; (–) = not detected; (+) = relative detection intensity

(Table 2). The maize *COB* gene probe hybridized to a 3.0 kb *Eco*RI fragment in all lines examined (Table 2).

The maize *ATP*A probe (Isaac et al. 1985 b) hybridized with the same relative intensity to two *Eco*RI fragments in all cytoplasms with the exception of IS1112C and M35-1 cytoplasms, in which only a single fragment was identified (Fig. 1 C). The fragments which hybridized were 5.6 and 3.9 kb in Kafir and IS2483C; 3.6 and 2.3 kb in Milo and IS12662C; 3.9 and 2.6 kb in 9E and IS7920C and 3.9 kb in IS1112C and M35-1

cytoplasm (Table 2). Maize *ATP*A probes specific to 5' or 3' coding sequence gave an identical hybridization pattern (not shown) which suggests that the *ATP*A open reading frame is present in two complete copies in Kafir, IS2483C, Milo, IS12662C, 9E and IS7920C cytoplasm.

The effect of varying nuclear genotype on MtDNA restriction digestion pattern and gene location

The effect of nuclear genotype on *Eco*RI and *Hind*III digestion pattern and mitochondrial gene location was examined. No differences in mtDNA restriction endonuclease digestion patterns or *Eco*RI fragment location of the four mitochondrial genes were observed for the various nuclear-cytoplasmic combinations of the six CMS cytoplasms with one exception. A difference was detected in the *Hind*III digestion pattern of mtDNA from the CMS and male fertile lines containing IS7920C cytoplasm. This restriction fragment polymorphism was evident when the *Hind*III digested mtDNA was hybridized with the ^{32}P-labelled 4.3 kb *Eco*RI fragment containing the *COX*I gene of Milo cytoplasm (Fig. 2). This probe hybridized with two approximately, 1.9 kb *Hind*III fragments from the male fertile line IS7920C nucleus in IS7920C cytoplasm, and only one 1.9 kb fragment from the CMS line Kafir nucleus in IS7920C cytoplasm. A 1.9 kb *Hind*III fragment was not detected in 9E cytoplasm (Fig. 2), of the same taxanomic race as IS7920C, indicating that mtDNA sequence heterogeneity between IS7920C and 9E cytoplasms exists in sequences homologous to this probe.

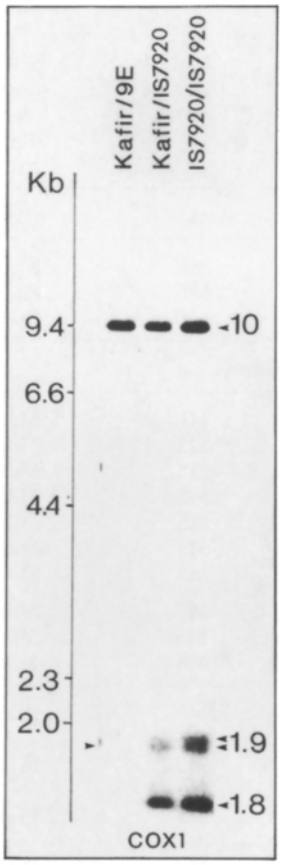

Fig. 2. Identification of *Hind*III generated mitochondrial DNA fragments of 9E and IS7920C cytoplasm which hybridize to the Milo *COX*I gene probe. Mitochondrial DNA from the nuclear-cytoplasmic combinations (nucleus/cytoplasm) Kafir/9E, Kafir/IS7920C and IS7920C/IS7920C was digested with *Hind*III, fractionated by electrophoresis in a 0.8% (w/v) agarose gel, transferred to nitrocellulose and hybridized with the ^{32}P-labelled pSM4.3 (containing the 4.3 kb *Eco*RI fragment of Milo mtDNA) and exposed to X-ray film. The size of the restriction fragments identified are indicated

The effect of nuclear genotype on the abundance of the 5.3 and 5.7 kb plasmid-like MtDNAs

MtDNA was isolated from seedling mitochondria, fractionated by electrophoresis in a 0.8% (w/v) agarose gel, stained with EtBr and photographed. As previously shown, two plasmid-like mtDNAs of 5.7 and 5.3 kb (Dixon and Leaver 1982), designated N1 and N2, respectively, (Pring et al. 1982), were detected in similar amounts in the CMS lines containing IS1112C and M35-1 cytoplasm (Kafir nucleus in IS1112C cytoplasm; Kafir nucleus in M35-1 cytoplasm; M35-1 nucleus in M35-1 cytoplasm) and the male fertile line containing IS1112C cytoplasm. These plasmid-like mtDNAs were present in very low amounts, or were undetectable in male fertile lines with M35-1 nucleus in M35-1 cytoplasm (Fig. 3), and not detected in the other cytoplasms examined (Table 2).

The effect of varying nuclear genotype on mitochondrial gene expression

Mitochondria were isolated from etiolated coleoptiles of the CMS and male fertile lines and allowed to synthesize proteins in the presence of L-[^{35}S]-methionine. The labelled translation products were fractionated by electrophoresis in a 16% (w/v) SDS-polyacrylamide gel and detected by autoradiography. When the spectrum of polypeptides synthesized by mitochondria from plants with a normal (male fertile) cytoplasm is used as a standard (i.e. Kafir (Fig. 4 A)), then specific variant polypeptides are detected among the translation products of mitochondria from CMS cytoplasms.

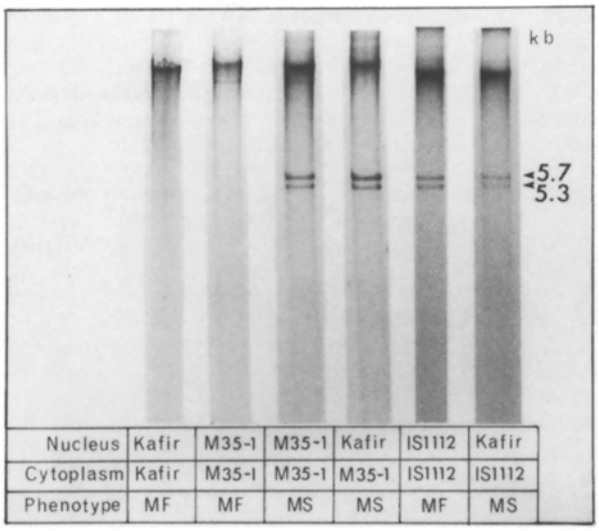

Fig. 3. Agarose gel electrophoresis of native mitochondrial DNA from various nuclear-cytoplasmic combinations of sorghum. Mitochondrial DNA was extracted and fractionated in 1% (w/v) agarose gels. Gels were stained with EtBr and photographed under UV light. The size of the plasmid-like mtDNAs are indicated in kb. MS = male sterile; MF = male fertile

As previously reported, mitochondria from Milo cytoplasm synthesize an additional 65 KD polypeptide which can be correlated with expression of the CMS phenotype (Dixon and Leaver 1982). The variant 65 KD polypeptide is synthesized in the fertile line Milo nucleus in Milo cytoplasm at less than 1% of the relative level in the CMS line Kafir nucleus in Milo cytoplasm (Fig. 4 B, C) as determined by densitometric tracing of the autoradiograph. Similar results were observed when the mitochondrial translation products of the CMS and male fertile lines containing IS12662C cytoplasm were examined (Table 2). In addition, we found that the variant 65 KD polypeptide is synthesized by 19 other CMS cytoplasms (Table 3).

Mitochondrial translation products of fertile and CMS lines containing IS1112C cytoplasm were examined. Mitochondria from the CMS line Kafir nucleus in IS1112C cytoplasm synthesize additional 12 KD and 82 KD polypeptides not detected among the translation products of Kafir cytoplasm (Fig. 4 A, E). Mitochondria from the male fertile line IS1112C nucleus in IS1112C cytoplasm synthesized the 12 KD polypeptide at a much reduced level but the 82 KD polypeptide at a

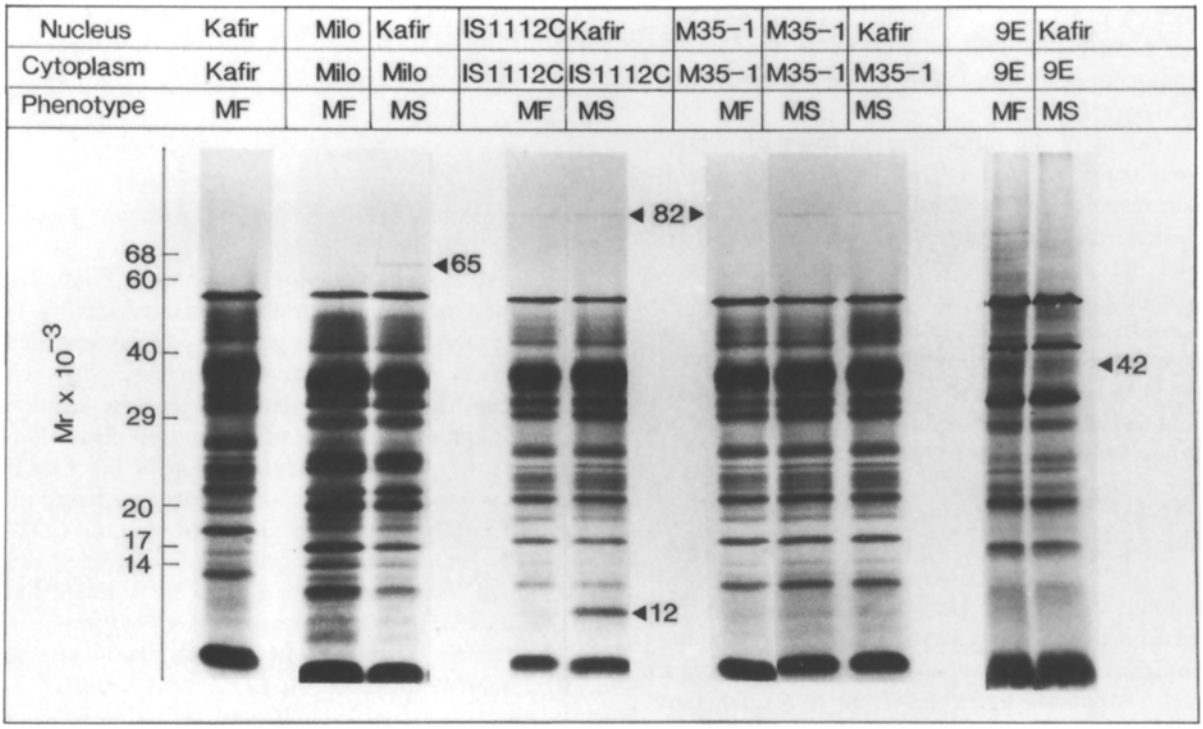

Fig. 4. Polypeptides synthesized by mitochondria isolated from various nuclear-cytoplasmic combinations of sorghum. Isolated mitochondria were incubated for 90 min in a medium containing ^{35}S-methionine. Mitochondrial polypeptides were fractionated by electrophoresis in 16% (w/v) SDS-polyacrylamide gels and labelled polypeptides were detected by autoradiography. Each gel track was loadedd with 5×10^5 cpm of trichloracetic acid insoluble radioactivity. The molecular weight (KD) of the variant polypeptides are indicated. MS = male sterile; MF = male fertile

similar level compared to the CMS line with IS1112C cytoplasm (Fig. 4 D, E). Thus, the synthesis of a variant 12 KD polypeptide correlates with the expression of the CMS phenotype in IS1112C cytoplasm.

Mitochondria from CMS lines with M35-1 cytoplasm synthesize an 82 KD polypeptide which is not detected among the translation products of mitochondria from Kafir cytoplasm (Dixon and Leaver 1982). Comparison of the mitochondrial translation products of the male fertile line M35-1 nucleus in M35-1 (A) cytoplasm, and two CMS lines with M35-1 (B) cytoplasm revealed that the variant 82 KD polypeptide is only detected in the CMS lines with M35-1 (B) cytoplasm (Fig. 4 F, G, H). Mitochondria from the CMS line synthesize the variant 82 KD polypeptide and possess two linear plasmid-like mtDNAs. Neither the 82 KD polypeptide nor the plasmid-like mtDNAs were detected in the male fertile line containing M35-1 (A) cytoplasm (Fig. 3, Fig. 4 F). Since this male fertile line had a mtDNA restriction pattern identical to that of Milo cytoplasm and possessed very low amounts of the 5.7 and 5.3 kb plasmid-like mtDNAs, in contrast to what was observed for the CMS lines with M35-1 (B) cytoplasm (Table 2), we are examining additional sources of male fertile M35-1 to confirm our analyses of this nuclear-cytoplasmic combination. Despite this, it appears that in IS1112C and M35-1 (B) cytoplasm the presence of the 5.3 and 5.7 Kb plasmid-like mtDNA molecules is correlated with the synthesis of the variant 82 KD mitochondrial translation product but not the CMS phenotype (Table 2).

Examination of the mitochondrial translation products from male fertile and CMS lines containing 9E cytoplasm revealed that a 42 KD polypeptide replaces a 38 KD polypeptide of Kafir cytoplasm (Fig. 4 A, I, J). We found that the 42 KD polypeptide is synthesized only by male fertile and CMS lines with the Nigerian cytoplasms 9E and IS7920C. Immunolabelling using an antiserum raised against yeast subunit I of cytochrome *c* oxidase (COI) has shown that the 42 KD polypeptide of 9E and IS7920C mitochondria is a variant form of COI (Bailey-Serres et al. 1986).

Discussion

The sorghum cytoplasms thus far surveyed can be classified into three groups based on restriction endonuclease digestion of chloroplast DNA, eleven groups based on restriction digestion of mtDNA and five groups based on synthesis of variant polypeptides by isolated mitochondria (Table 3) (Pring et al. 1982; Conde et al. 1982; Bailey-Serres, presented here). In addition, the lines IS1112C and M35-1 can be distinguished by the presence of two linear plasmid-like mtDNA molecules of 5.7 and 5.3 kb (Dixon and Leaver 1982; Pring et al. 1982).

Table 3. Classification of sorghum cytoplasms by molecular analyses. Additional cytoplasms examined by in organello mitochondrial protein synthesis were IS7502C, IS2266C, IS3579C, SA385 – 1, 2, 3, 4, 5, 6, in Kafir nuclear background, Early White Milo, Standard Yellow Milo, Dwarf Yellow Milo – all synthesized an additional 65 KD dalton polypeptide

Cytoplasm[a]	Variant polypeptide (KD)	MtDNA group	CpDNA group	Plasmid like MtDNA (kb)
Normal cytoplasms				
Kafir	None	10	2	none
Martin	None	10	–	
IS2483C	None	11	–	
Millo Blanco	None	6	2	
CMS cytoplasms				
Milo	65	1	1	
IS6705C	65			
IS2801C	65			
IS3063C	65			
KS34	65			
KS38	65			
KS39	65			
KS35	65	9		
KS36	65			
KS37	65			
IS12662C	65	2	–	
IS1056C	65	5	2	
IS1112C	82, 12	3	2	5.7, 5.3
M35-1 (B)	82	4	–	5.7, 5.3
9E (IS17218)	42 COI	7	3	none
IS7920C	42 COI	8	3	none

[a] Pring et al. 1982

We have shown by hybridization of ^{32}P-labelled gene probes to restricted mtDNA that variability in mtDNA organization of *Sorghum bicolor,* as revealed by differences in restriction endonuclease digestion patterns, can be due to differences in genomic location and copy number of specific mitochondrial genes. Differences in the *Eco*RI fragment location of the *COX*II and *COB* genes were not detected. However, based on the *Eco*RI mtDNA fragments identified with the *COX*I and *ATP*A gene probes the cytoplasms examined can be classified into four groups (Table 2). A single but variable *Eco*RI fragment location of *COX*I was observed in each cytoplasm. In contrast, two variable *Eco*RI fragment locations of *ATP*A were detected in each cytoplasm except IS1112C and M35-1, in which a single *Eco*RI fragment location of *ATP*A was identified.

It has been shown that changes in nuclear genotype affect the restriction fragment location and abundance of *COX*I in S cytoplasm maize (Isaac et al. 1985a; Leaver et al. 1985). Similar evidence for nuclear control of mitochondrial genome

organization in sorghum was not obtained except in one case. In IS7920C cytoplasm we observed a difference between the male fertile and CMS lines when *Hind*III digested mtDNA was probed with a 4.3 kb fragment containing the Milo *COX*I gene. This difference in genome organization could be due to nuclear control of mtDNA organization or a specific alteration which occurred during the production of the CMS line.

We observed that nuclear genotype can have specific effects on the products of in organello mitochondrial protein synthesis when certain cytoplasms are transferred from their natural nuclear background to a non-fertility maintaining nuclear background. Nuclear regulation of mitochondrial gene expression was evident since variant mitochondrial polypeptides were synthesized at high levels in CMS and at low levels by male fertile nuclear-cytoplasmic combinations with Milo, IS12662C, IS1112C and M35-1 cytoplasms (Table 2). Similarly, in the absence of nuclear fertility alleles, the synthesis of variant mitochondrial polypeptides and the expression of the CMS phenotype is observed in maize, sugarbeet, field bean, tobacco and petunia (Leaver and Gray 1982; Boutry et al. 1984; Hanson and Conde 1985).

Recent molecular evidence suggests that variant mitochondrial polypeptides arise from mtDNA rearrangement events which result in the formation of novel mitochondrial open reading frames which are transcribed and translated. We have demonstrated that in 9E cytoplasm sorghum, mitochondrial genome rearrangements led to the relocation of the *COX*I gene, extension of the open reading frame and synthesis of a larger subunit I polypeptide (Bailey-Serres et al. 1986). In T cytoplasm maize, multiple genome rearrangement events resulted in the formation of an open reading frame which encodes a 13 KD polypeptide, which is expressed at high levels in CMS T and low levels in nuclear restored T cytoplasm maize (Dewey et al. 1986). Such genome rearrangement events may result in the varying mitochondrial genotypes observed within sorghum and other plant species.

Since mitochondrial biogenesis and function is dependent upon the coordinated expression of nuclear and mitochondrial genes, one can speculate that specific nuclear genes may have evolved to complement individual mitochondrial genotypes. When a cytoplasm is transferred to a foreign nuclear background, the absence of specific alleles may result in the expression of cytoplasmic mutations such as male sterility. Nuclear mutations which have co-evolved with mitochondrial genome rearrangements may suppress or compensate for the expression of variant mitochondrial polypeptides. Nuclear mutations which compensate for mitochondrial mutations have been described in yeast (Dujardin et al. 1980; Groudinsky et al. 1981; Labouesse et al. 1985; Kruszewka and Szczesniak 1985). In higher plants, nuclear fertility alleles may suppress or compensate for specific mitochondrial mutations which are detrimental during microspore development, a developmental stage associated with increased levels of respiration and mitochondrial biogenesis. In support of this hypothesis, it has been observed that maternally inherited reversion to male fertility in S and T cytoplasm maize is correlated with specific changes in mitochondrial genome organization (Schardl et al. 1984; Kemble et al. 1983) and loss of the characteristic variant mitochondrial polypeptides (Dixon et al. 1982). It appears that these mtDNA rearrangements circumvent the requirement of a given cytoplasmic genotype for specific nuclear fertility alleles. Thus, the further examination of the CMS phenotype may reveal specific nuclear-mitochondrial interactions which are required for normal plant development.

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