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Restriction endonuclease analysis of mitochondrial DNA from sorghum with fertile and male-sterile cytoplasms*

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Summary. Mitochondrial DNA from four paired (fertile and male-sterile) lines and six isocytoplasmic strains of sorghum (*Sorghum bicolor* (L.) Moench) were fragmented by endonucleases and their electrophoretic patterns were examined. Cytoplasmic male sterile lines differed from their male-fertile counterparts consistently. Among the isocytoplasmic strains, KS 36A (*S. verticilliflorum* cytoplasm), KS 38A (*S. conspicum* cytoplasm), and KS 39A (*S. niloticum* cytoplasm) showed minor differences from the other strains. Results suggest that restriction endonuclease patterns are useful in detecting differences in mitochondrial genomes.

Key words: *Sorghum bicolor* (L.) Moench – Agarose gel electrophoresis – isocytoplasmic strains

Introduction

Evidence obtained by molecular techniques for examining cytoplasmic genomes directly suggests that the genetic determinants controlling cytoplasmic male sterility (CMS) are carried by the mitochondrion (Leaver and Gray 1982). In sorghum (*Sorghum bicolor* (L.) Moench), almost all commercial F_1 hybrids and their female parents have the A_1 (milo) type male-sterility-inducing cytoplasm. Analysis of mitochondrial DNA (mtDNA) with restriction endonucleases showed that cytoplasmic male sterility in sorghum was associated with alterations in mtDNA (Conde and Pring 1978; Conde et al. 1982; Hanson and Conde 1985; Pring et al. 1982; Pring 1983). In addition, plasmid-like DNAs were present in CMS sorghums (Chase and Pring 1986; Pring 1983). To confirm alterations in mtDNA restriction patterns and the presence of plasmid-like DNA in CMS material, it is desirable to examine different CMS lines, their male-fertile counterparts, and available isocytoplasmic germplasm lines developed by Ross and Hackerott (1972). A system relating CMS and its restriction pattern in sorghum is necessary to document the utility of the restriction endonuclease systems in searching for and identifying additional CMS lines for hybrid production.

Our paper describes the variation of restriction endonuclease patterns of mtDNA from paired (CMS and male-fertile) grain sorghum lines and from six isocytoplasmic lines with a Combine Kafir 60 nucleus, in an attempt to establish a relationship between mtDNA restriction patterns for male-sterile cytoplasms.

Materials and methods

Plant materials

We studied the following: four pairs of grain sorghum lines – KS 5A and 5B, KS 45A and 45B, Redlan A and B, and Martin A and B; six isocytoplasmic lines – KS 34A through 39A, which respectively possess cytoplasms from *S. arundinaceum* (SA 1471 and PI 258806), *S. verticilliflorum, S. sudanense, S. conspicuum,* and *S. niloticum* (Ross and Hackerott 1972); and a forage sorghum – Sumac.

Ten grams of seeds from each source were sterilized (50% Chlorox [2.7% NaOCl] for 10 min with agitation), rinsed with tap water (1 h), submerged in 70% ethanol (2 min), and rinsed again with sterile water. The sterilized seeds were placed in sterile Petri dishes containing two layers of sterile moist blotting paper. The petri dishes were sealed and kept in dark conditions for 1 week at 24°C. The etiolated mesocotyl and coleoptile were cut with a pair of scissors and weighed before homogenization.

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Isolation of mitochondrial DNA

Mitochondria were isolated in a cold room at 4 °C (Kemble et al. 1980). Following the DNase treatment to remove nuclear DNA and purification with sucrose gradient, the mitochondrial pellet was resuspended in 2 ml of lysis buffer (0.05 *M* Tris HCl [pH 8], 0.01 *M* EDTA [pH 8], 2% Sarcosyl, 0.02% autodigested Proteinase K), and incubated at 37 °C for 1 h with gentle shaking (40 rpm).

The lysate was deproteinized by several phenol/chloroform extractions. The mtDNA was precipitated with two volumes of 100% ethanol and washed with 70% ethanol. The mtDNA was dissolved in 30–80 l of 10 m*M* Tris HCl (pH 8) and 5 m*M* EDTA (pH 8), and purified by adding spermine to the DNA sample until a concentration of 100 m*M* was obtained. The solution was mixed gently, kept at 4 °C for 15 min, and then centrifuged at 14,000 × g at 4 °C for 20 min. The supernatant was removed. The DNA pellet was vacuum-dried for 7 min and resuspended in the original volume of 10 m*M* Tris HCl (pH 8) and 5 m*M* EDTA (pH 8).

Restriction endonuclease digestion

The restriction enzymes, EcoRI, HindIII, and BamHI (Bethesda Research Lab, Inc.), were used according to supplier's recommendations. Five to six grams of mtDNA were digested in 20 l reaction mixture with 20–40 units of restriction enzyme and incubated at 37 °C for 6 h. The digestion was determined by adding 5 l loading dye (0.25% bromophenol blue, 0.25% xylene cyanol, 25% Ficoll [type 400]) (Maniatis et al. 1982). The mt DNA was immediately stored at −20 °C.

Agarose gel electrophoresis

Digested mtDNA fragments were separated by electrophoresis in a 0.7% agarose horizontal slab gel at 3 v/cm for 17 h at 22 °C in TBE buffer (0.089 *M* Trisma base, 0.089 *M* Boric acid, 0.002 *M* EDTA). The gel was stained with 0.6 g/ml ethidium bromide for 20 min and destained for 20 min in deionized water. The gel was placed on top of a UV transilluminator and photographed through Kodak Wratten Nos. 9 and 23 filters with Type 55 P/N Polaroid film.

Results and discussion

The EcoRI restriction patterns of Sumac, KS 5B, Redlan B, Martin B and KS 45B, their four male-sterile counterparts, and KS 34A are shown in Fig. 1. Compared with patterns of the male-fertile lines, mtDNA restriction patterns of CMS lines have an extra band near 6.4 kb and are missing two bands just below 6.4 kb. The restriction patterns of all CMS lines are identical to each other, which is not surprising because they are all milo-type cytoplasms. Sumac and KS 5B had the same banding patterns, which was expected since KS 5B is a selection from Sumac. Restriction patterns of Redland B, Martin B and KS 45B are identical to each other, so they may have had the same female parent. Cytoplasms of Sumac and KS 5B differed from those of Redland B, Martin B, and KS 45B, as shown by the three small arrows and a large arrow between 4.3 and 9.4 kb and a medium arrow between 2.3 and 4.3 kb.

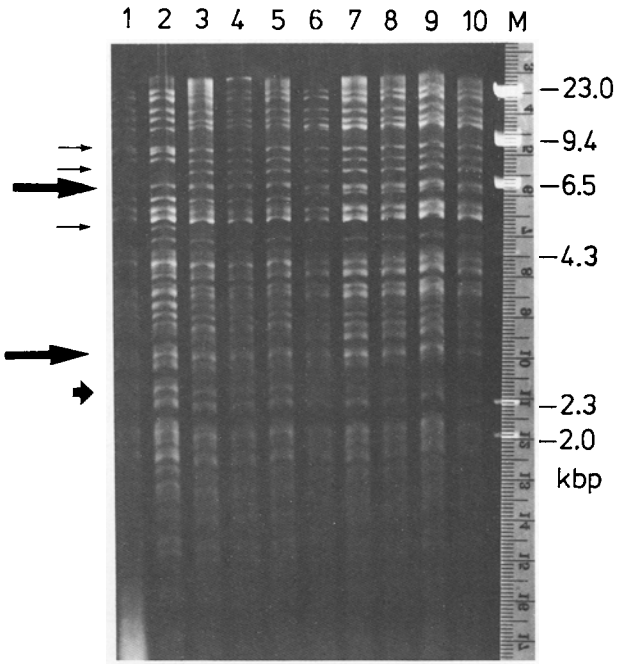

Fig. 1. Agarose gel electrophoretic patterns of EcoRI digestion of mtDNA from male-fertile (*B lines, lanes 1–5*) and cytoplasmic male-sterile (*A lines, lanes 6–10*) lines. M: molecular weight marker from bacteriophage lambda DNA; *lane 1:* Sumac; *lane 2:* KS 5B; *lane 3:* Redlan B; *lane 4:* Martin B; *lane 5:* KS 45B; *lane 6:* KS 5A; *lane 7:* Redlan A; *lane 8:* Martin A; *lane 9:* KS 45A; *lane 10:* KS 34A

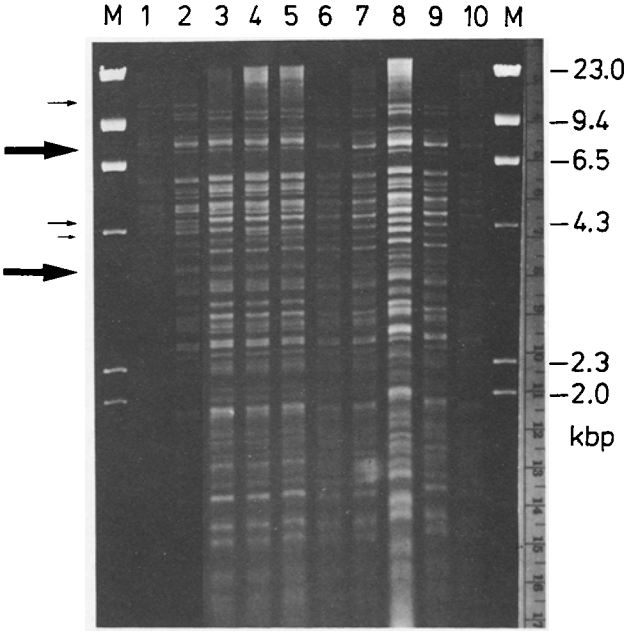

Fig. 2. Agarose gel electrophoretic patterns of Hind III digestion of mtDNA from male-fertile (*B lines, lanes 1–5*) and cytoplasmic male-sterile (*A lines, lanes 6–10*). M: molecular weight marker from bacteriophage lambda DNA; *lane 1:* Sumac; *lane 2:* KS 5B; *lane 3:* Redlan B; *lane 4:* Martin B; *lane 5:* KS 45B; *lane 6:* KS 5A; *lane 7:* Redlan A; *lane 8:* Martin A; *lane 9:* KS 45A; *lane 10:* KS 34A

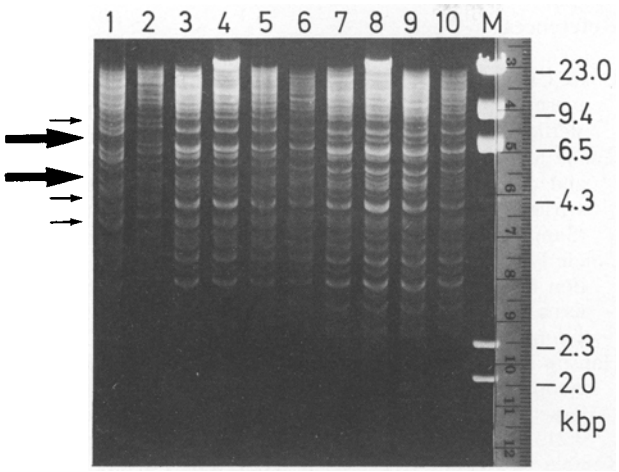

Fig. 3. Agarose gel electrophoretic patterns of BamHI digestion of mtDNA from male-fertile (*B lines, lanes 1–5*) and cytoplasmic male-sterile (*A lines, lanes 6–10*) lines. M: molecular weight marker from bacteriophage lambda DNA; *lane 1:* Sumac *lane 2:* KS 5B; *lane 3:* Redlan B; *lane 4:* Martin B; *lane 5:* KS 45B; *lane 6:* KS 5A; *lane 7:* Redlan A; *lane 8:* Martin A; *lane 9:* KS 45A; *lane 10:* KS 34A

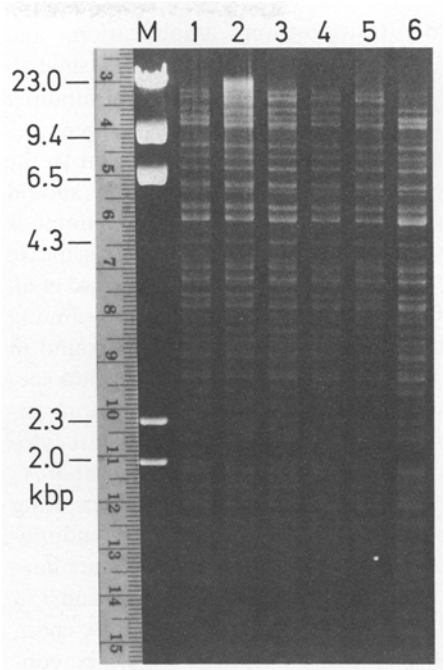

Fig. 4. Agarose gel electrophoretic patterns of EcoRI digestion of mtDNA from isocytoplasmic CMS strains. M: molecular weight marker from bacteriophage lambda DNA; *lane 1:* KS 34A (with cytoplasm from *S. arundinaceum* SA 1741); *lane 2:* KS 34A (with cytoplasm from *S. arundinaceum* PI 258806); *lane 3:* KS 36A (with cytoplasm from *S. verticilliflorum* PI 208190); *lane 4:* KS 37A (with cytoplasm from *S. sudanense* PI 247722); *lane 5:* KS 38A (with cytoplasm from *S. conspicuum* PI 155140); *lane 6:* KS 39A (with cytoplasm from *S. niloticum* PI 155140)

The mtDNA digested by endonuclease HindIII, like EcoRI, showed that the CMS lines (KS 5A, Redlan A, Martin A, KS 45A, and KS 34A) were identical in banding patterns, but differed from those of their male-fertile counterparts (Fig. 2, large arrows). Sumac and KS 5B were identical to each other and had two more bands than other male-fertile lines (Fig. 2, medium arrows). Restriction patterns of Martin B and KS 5B were identical to each other, but each had one more band than Redlan B (Fig. 2, short arrow).

The BamHI digestion of mtDNA produced more large DNA fragments than the EcoRI and Hind III digestions. The large fragments were concentrated between 9.4 and 23 kb (Fig. 3) and were difficult to separate even with 0.7% agarose gel. The restriction patterns showed that CMS lines were the same, except that KS 5A, Redlan A, and KS 45A had one more band than the others (Fig. 3, lanes 6, 7, 9) between 6.5 and 9.4 kb (small arrow), and KS 5A had one less band near 4.3 kb (Fig. 3, line 6). However, patterns of CMS lines differ from those of male-fertile lines by having two more bands, one just above 6.5 kb and the other near 5.3 kb (Fig. 3, lanes 6–10, large arrows). Sumac and KS 5B had restriction patterns similar to each other, but had two more bands near 4.3 kb (Fig. 3, lanes 1 and 2, small arrow) and were missing two bands near 4.0 kb (Fig. 3, lane 1 and third small arrow from top) compared to other male-fertile lines.

Although sources of cytoplasms were different among CMS lines (KS 34A through 39A), their mtDNA restriction patterns as revealed by EcoRI digestion showed only minor differences (Fig. 4, lanes 1 through 6). An extra but faint band occurred in KS 39A near 9.4 kb (Fig. 4, lane 6); also an extra but very faint band appeared near 9.5 kb in KS 36A and 38A (Fig. 4, lanes 3 and 5).

In corn, CMS cytoplasms are characterized by small linear molecules of DNA, such as the S-1 and S-2 of 6.2 and 5.2 kb, respectively, in the S cytoplasm (Pring 1983). In sorghum, plasmid-like DNAs, N-1 and N-2, were found in one alternative CMS cytoplasm (IS1112C) (Pring et al. 1982; Pring 1983). However, it is not known if those molecules would control the expression of CMS in a manner similar to S-1 and S-2 in maize. In this experiment, no plasmid-like DNAs were observed in all the CMS materials we tested. Apparently, N-1 and N-2 molecules are unique to the IS1112 cytoplasm.

Cytoplasmic male sterility is a nonlethal mutation of the mitochondrial genomes in higher plants (Hanson and Conde 1985). The high frequency of simultaneous occurrence of CMS and fertile cytoplasms within many species reveals the fluidity of the cytoplasmic genome, which may undergo frequent alterations through the processes

of rearrangement, transposition, amplification, and mutation. With the molecular approach, cytoplasmic alterations having indistinguishable reactions to modifier genes can be distinguished, if their cytoplasmic genomes exhibit restriction site heterogeneity. In addition to the mitochondrial genomes, chloroplast genomes should also be examined closely. The mitochondrial genome is apparently less conserved and more heterogeneous in size among species than the chloroplast genomes (Ward et al. 1981). Restriction fragment size differences among mtDNA in fertile and sterile cytoplasms were found in maize, sorghum, sugarbeet, and in many other plant species (Hanson and Conde 1985), whereas restriction patterns of chloroplast DNA (ctDNA) in seven isocytoplasmic sorghums were indistinguishable from that of A_1 sorghum cytoplasm (Conde et al. 1982). However, using two-dimensional electrophoresis, differences in endonuclease fragments between CMS and their maintainer lines were discovered in maize, wheat, and rape (Li and Liu 1983). Obviously, further experiments in ctDNA endonuclease digestion patterns in sorghum should be conducted to correlate functioning of cytoplasmic-genetic information with aberrant reproductive development, because different types of male-sterility-inducing cytoplasms are available in sorghum. Likewise, mtDNA analysis should also be used on A_2 and other types of CMS systems with a standard set of endonucleases, and on altered and evolutionally diverged mitochondrial genomes in related species, to reveal the degree of changes in those genomes. Thus, CMS systems in plant species could offer a useful tool for analysis of their functioning and variation as an adaptive mechanism to the ever-changing dynamic environment.

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