Theor Appl Genet (1991) 81:793–799

Characterization of pearl millet mitochondrial DNA fragments rearranged by reversion from cytoplasmic male sterility to fertility *

R.L. Smith ** **and M.K.U. Chowdhury**

Departments of Agronomy and Vegetable Crops, University of Florida, Gainesville, FL 32611, USA

Received April 9, 1990; Accepted October 30, 1990
Communicated by P.L. Pfahler

Summary. Cloned pearl millet [*Pennisetum glaucum* (L.) R. Br.] mitochondrial (mt) DNA fragments rearranged by spontaneous reversion from cytoplasmic male sterility (cms) to fertility were characterized by restriction mapping, hybridization with maize mt genes, and transcription analyses. The clones characterized were a 4.7-kb fragment found only in the male-sterile cytoplasm and lost upon reversion to fertility, a 10.9-kb fragment found in all cytoplasms and not changed by reversion, a 13.6-kb fragment found in the male-sterile and -fertile normal cytoplasms and lost in seven of the eight revertants studied, and a 9.7-kb fragment not found in the male-sterile cytoplasm but produced by reversion from male sterility to fertility. The restriction maps verified that the four cloned pearl millet fragments contained two sets of repeated sequences, one on the 4.7-, 10.9-, and 13.6-kb fragments, the other on the 13.6- and 9.7-kb fragments. The *rrn18*, *rrn5*, and *coxI* genes were located in the repeated regions of the 4.7-, 10.9-, and 13.6-kb cloned fragments. The correlation of reversion (eight independent events) with the loss of fragments containing the *rrn18*, *rrn5*, and *coxI* genes suggests that those lost fragments and their gene content could be responsible for the expression of cms. Transcriptional analyses using both Northern blots and end-labeled mtRNA probes verified that transcripts homologous to the *rrn18* and *coxI* genes were present in pearl millet total mtRNA. However, no transcript differences were detected among cms, revertant, and fertile normal cytoplasms, suggesting that the reversion process involves mutational changes that may not affect transcript size. Transcript analyses indicated that the 10.9-kb clone contained an unidentified gene on the end opposite the *rrn18* gene; however, since it was present in all cytoplasms, it is not believed to be involved in cms.

Key words: Cytoplasmic male sterility – Hybridization patterns – Mitochondria – *Pennisetum glaucum* – Reversion

Introduction

The cytoplasmic male sterility (cms) trait is important commercially to provide pollination control for production of F_1 seed that, in turn, produce superior-yielding crops. Pearson (1981) reviewed the use of cms in crop production, and Hanson and Conde (1985) reviewed the molecular study of cms. Basic molecular research on cms is most advanced in the maize cms-T and petunia systems. However, variability in the expression of cms, rate of mutation (reversion) to fertility, and interaction with nuclear restorer genes of the various cms systems suggest that basic molecular mechanisms differ in the different systems (Hanson and Conde 1985; Pearson 1981). Studies of other cms systems may help determine whether or not there are unifying determinants of molecular mechanisms of cms.

In both the cms-T maize and petunia systems, reversion to fertility is accompanied by mitochondrial genome rearrangements, resulting in a loss or modification of a DNA fragment, and in both systems the lost fragment contains a chimeric gene. In maize, *urf13-T*, which encodes a 13-kDa mitochondrial membrane protein (Forde and Leaver 1980; Dewey et al. 1987) associated with cms, and susceptibility to the fungal pathogen, *Bipolaris maydis* (Dewey et al. 1988), is lost (Dewey et al. 1986) or

* Florida Agricultural Experiment Station Journal Series No. R-00035

** To whom correspondence should be addressed

modified (Wise et al. 1987). The petunia chimeric gene, *Pcf*, which encodes a fusion peptide believed to be responsible for cms (Young and Hanson 1987), is lost upon reversion to fertility. Even though genes believed to cause cms in both maize and petunia have been identified, the molecular mechanisms of cms remain undefined.

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is an excellent species for the study of cms mechanisms because of the diversity of cms types. The availability of spontaneous fertile revertants from those male-sterile cytoplasms (Smith and Chowdhury 1989) permits the study of cosegregation of cms and fertility with mitochondrial genomic rearragements.

Earlier we reported identifying and cloning fragments that were rearranged by reversion of the S-A1 male-sterile cytoplasm to fertility (Smith et al. 1987). Four PstI fragments were cloned: a 4.7-kb fragment found only in the male-sterile mitochondria, a 9.7-kb fragment found only in the eight reverted cytoplasms, a 13.6-kb fragment found in the sterile, one revertant and the maintainer (normal) cytoplasms. The fourth clone contained at 10.9-kb fragment found in all cytoplasms (not rearranged by reversion). However, it contained sequences repeated in the 4.7- and 13.6-kb clones fragments. The loss of the 4.7-kb and the gain of the 9.7-kb fragments in all eight revertants suggest that genes located upon those fragments may be involved in cms determination.

In this publication, we report characterization of the four cloned pearl millet mtDNA fragments described above that were rearranged during spontaneous reversion from cms to fertility. The fragments were mapped and gene content was determined by hybridization to known maize mitochondrial gene probes. Results are also reported of transcription studies of those cloned mtDNA fragments, using end-labeled pearl millet mtRNA probes on Southern blots, and pearl millet and maize gene probes on pearl millet mitochondrial Northern blots.

Materials and methods

Source and analysis of mtDNA

Pearl millet mtDNA was isolated from the S-A1 male-sterile cytoplasm of Tift 23DA (labeled A1 in the figures), eight fertile spontaneous revertants of S-A1 (the two shown in the figures are labeled R1 and R2), and normal fertile cytoplasm of the inbred line Tift 23DB (labeled B1 in the figures). Tift 23DB is used as pollinator to maintain the CMS line, Tift 23DA, so they have identical nuclear backgrounds, but different cytoplasms. Refer to Fig. 1 for the fragment content of the above pearl millet lines. mtDNA isolation methods were published earlier (Smith et al. 1987). Plasmid DNA was isolated by the method of Holmes and Quigley (1981) from the clones of S-A1 rearranged by reversion to fertility. Restriction endonuclease digestions, electrophoresis, ethidium bromide staining, and photography were conducted as described by Chase and Pring (1986). HindIII-digested Lambda DNA and HaeIII-digested Phi X 174 DNA were used as molecular size markers, and restriction fragment sizes were calculated using the computer program of Schaffer and Sederoff (1981). DNA was transferred (Blotted) to Nytran membrane (Schleicher and Schuell, Inc.) as described by Maniatis et al. (1982).

Maize gene probes and hybridization

The following cloned maize mitochondrial genes were used as probes: cytoplasm *c* oxidase subunit I (*coxI*) (Isaac et al. 1985) and subunit II (*coxII*) (Fox and Leaver 1981), F_1-F_0 ATPase subunit alpha (*atpA*) (Braun and Levings 1985), subunit 6 (*atp6*) (Dewey et al. 1985a), and subunit 9 (*atp9*) (Dewey et al. 1985b); and the 26S (*rrn26*) (Dale et al. 1984) and 18S-5S (*rrn18-rrn5*) (Chao et al. 1983, 1984) ribosomal RNA genes. Additional maize clones containing sequences of ORF13 and ORF25 (Dewey et al. 1986) were also used as probes. The maize clones were supplied by C. S. Levings, III, North Carolina State University, Raleigh/NC, and D. R. Pring, University of Florida, Gainesville/FL. Probes were made using the maize clones or gene inserts of the clones cut from their vectors by restriction with the appropriate endonucleases, fractionated by electrophoresis, and then recovered from the gel using the NA45 membrane and the method of Schleicher and Schuell, Inc. The maize inserts were labeled with ^{32}P by the random primer extension method of Feinberg and Vogelstein (1983), and DNA hybridizations were conducted according to the methods described by Chase and Pring (1986). Hybridized blots were washed twice with $3 \times$ SSC ($1 \times$ SSC = 0.15 *M* NaCl, 0.015 *M* sodium citrate, pH 7.5) for 15 min and once with $0.3 \times$ SSC for 15 min, all at 65 °C. Then the blots were autoradiographed.

Mitochondrial RNA isolation

Total mitochondrial RNA (mtRNA) was isolated from mitochondria obtained from 6-day-old seedlings as described above. The mitochondria were lysed with 6 *M* guanidine isothiocyanate, extracted first with equal volumes of phenol, then chloroform, and finally purified by $CsCl_2$ centrifugation as described by Wise et al. (1987). The mtRNA was resuspended in water, made 1.6 *M* in ammonium acetate, and precipitated with 2 vol ethanol. The RNA was stored in the ethanol until needed, then recovered by centrifugation.

RNA electrophoresis, blotting, and hybridization

mtRNA was electrophoresed and Northern blots were hybridized as described by Wise et al. (1987). mtRNA was end-labeled by first hydrolyzing in a buffer containing 5 m*M* glycine, 100 μ*M* spermidine, and 10 μ*M* ethylene diamine tetraacetate (EDTA), pH 9.5, at 90 °C for 10 min to generate 5'-OH ends. The ^{32}P-label was then added by the T4 polynucleotide kinase reaction in 50 m*M* TRIS-HCl (pH 9.5), 10 m*M* $MgCl_2$, and 5 μ*M* dithiothreitol (DTT). The reaction was conduced for 30 min at 37 °C using 1 unit of enzyme, 1 μg of mtRNA, and 30 μCi of gamma ^{32}P-labeled ATP in a reaction volume of 16–18 μl. Unicorporated nucleotides were removed by Sephadex chromatography. An RNA ladder ranging in size from 9.5 to 0.3 kb (Bethesda Research Laboratories, Inc.) was used to estimate the size of the transcripts.

Results

mtDNA hybridization analyses

The maize probes were used to hybridize Southern transfers of PstI-digested cms, fertile revertant, and normal mtDNAs to determine whether pearl millet gene se-

quences similar to those of the maize gene probes were located on the fragments rearranged by reversion. Of the nine probes used, seven gave uniform hybridization patterns across the pearl millet cytoplasms, and only the *rrn18-rrn5* and *coxI* probes gave differential hybridization patterns across the cytoplasms. The *rrn18-rrn5* probe (Fig. 1 A) hybridized to the 4.7-, 10.9-, and 13.6-kb fragments. In addition, that probe hybridized to a 10.0-kb fragment (found only in the normal cytoplasm).

The *coxI* probe (Table 1 B) hybridized to the same four fragments as the *rrn18-rrn5* probe, i.e., the 4.7-, 10.9-, 10.0-, and 13.6-kb fragments. In addition, the 3.8-kb fragment found in all cytoplasms hybridized to the *coxI* probe but not to the *rrn18-rrn5* probe. Of the fragments mentioned, the 10.0-kb fragment in the normal cytoplasm and the 3.8-kb fragment found in all cytoplasms are not cloned. The 4.7-, 9.7-, 10.9-, and 13.6-kb fragments are cloned.

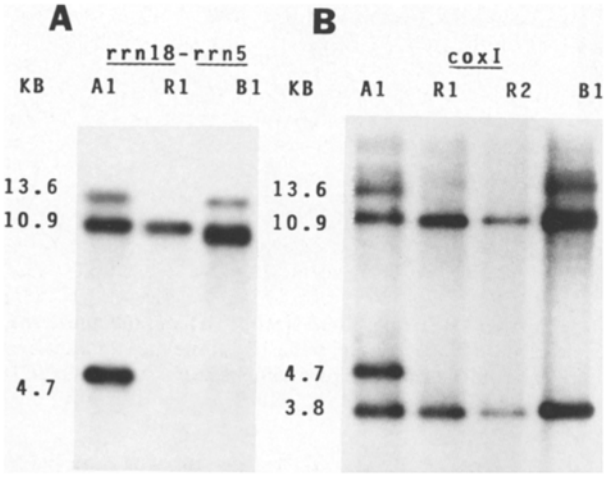

Fig. 1 A and B. Autoradiograph of Southern blots of PstI-digested mtDNA from cms Tift 23DA (A1), fertile revertant (R1 and R2), and the fertile normal Tift 23DB (B1) lines hybridized to maize mitochondrial genes *rrn18-rrn5* (**A**) and *coxI* (**B**), showing rearranged fragments containing those genes. Fragment sizes are given in kilobases (kb)

Clone mapping and hybridization

The restriction maps of the 13.6-, 10.9-, 9.7-, and 4.7-kb cloned pearl millet fragments are shown in Fig. 2. The restriction patterns on the right of the restriction maps of the 4.7-, 10.9-, and 13.6-kb fragments are identical, and left restriction patterns of the 13.6- and the 9.7-kb fragments are also identical.

Southern blots of the four cloned pearl millet fragments restricted with PstI plus BamHI, SmaI, or XhoI endonucleases (Fig. 3 B) were hybridized to the *rrn18-rrn5* and *coxI* maize gene probes. Those probes hybridized to sequences on the 4.7-, 10.9-, and 13.6-kb pearl millet clones, but not to the 9.7-kb clone. In the 4.7-kb clone, the *rrn18-rrn5* probe, consisting of the insert only (Fig. 3 A), hybridized to 1.0- and 3.7-kb PstI-XhoI fragments, a 0.5-kb SmaI fragment (PS lane), and 2.0- and 2.2-kb PstI-SmaI fragments. In the 10.9-kb clone, the *rrn18-rrn5* probe hybridized to a 1.0-kb PstI-XhoI fragment, a 4.9-kb XhoI fragment (PX lane), 0.5- and 8.0-kb SmaI fragments (PS lane), a 2.2-kb PstI-SmaI fragment, and a 4.8-kb PstI-BamHI fragment. In the 13.6-kb clone, the *rrn18-rrn5* probe hybridized to a 1.0-kb PstI-XhoI fragment, a 7.4-kb XhoI fragment (PX lane), 0.5- and

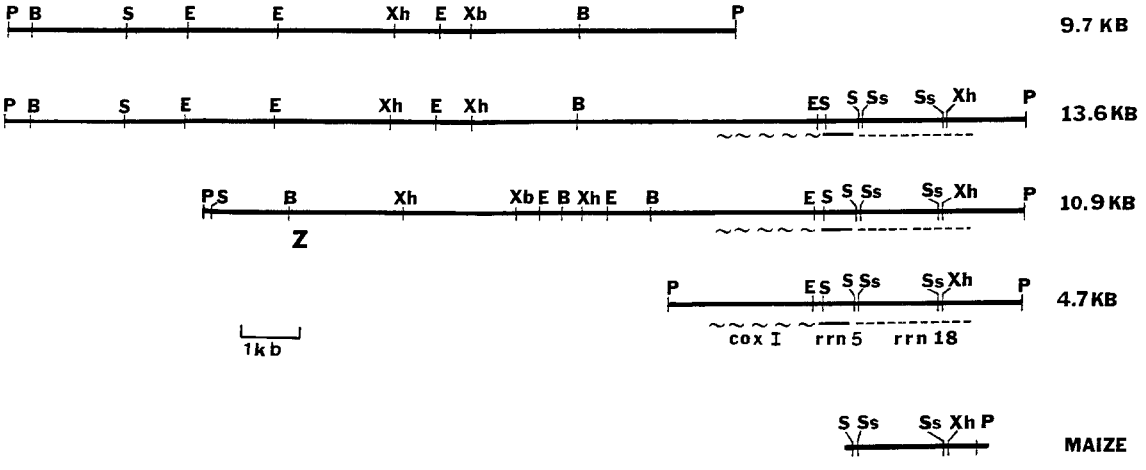

Fig. 2. Restriction maps of cloned pearl millet fragments rearranged by reversion, i.e., 4.7-kb fragment found only in the male-sterile cytoplasm and lost upon reversion, 13.6-kb fragment found in the male-sterile cytoplasm and normal line but lost in seven of eight revertants, and 9.7-kb fragment found only in the revertants. The 10.9-kb fragment found in all lines and not rearranged by reversion was included because it contained repeated sequences found on the 4.7- and 13.6-kb fragments. The restriction map of the maize *rrn18* gene shows similarity to the pearl millet *rrn18* sequence. The locations of the pearl millet *coxI*, *rrn6*, and *rrn18* genes are noted on the three clones; however, their sizes are only approximate. The region labeled Z contains an unidentified gene. B = BamHI, E = EcoRV, P = PstI, S = SmaI, Ss = SstII, Xb = XbaI, and Xh = XhoI

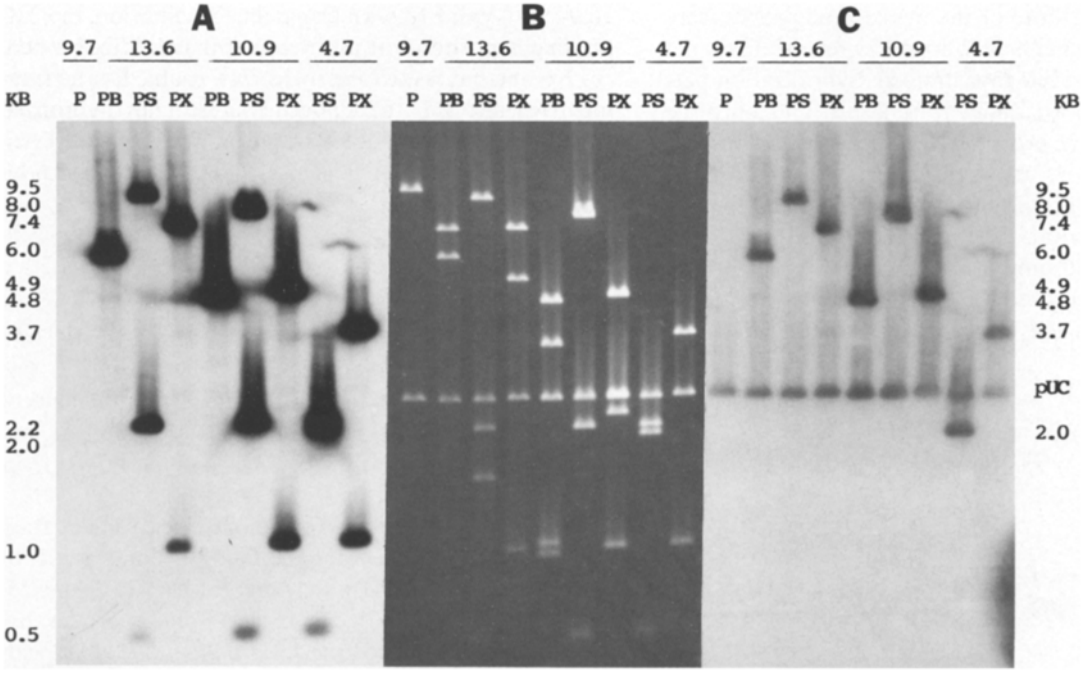

Fig. 3A–C. Hybridization of doubly digested mitochondrial clones with maize *rrn18-rrn5* gene insert probe (**A**) and the maize *coxI* clone probe containing pUC (**C**) shows the location of those genes on the pearl millet clones. Center panel (**B**) shows the ethidium-bromide-stained gel. Sizes of hybridized fragments are given on the *left* and *right* of the hybridized blots in kilobases; location of pUC18 is designated with pUC. Numbers at the *top* identify the clones. P = PstI, B = BamHI, S = SmaI, X = XhoI

9.5-kb SmaI fragments (PS lane), a 2.2-kb PstI-SmaI fragment, and a 6.0-kb PstI-BamHI fragment. The hybridization data indicated that the pearl millet *rrn18-rrn5* genes were located on the repeated sequences of the 13.6-, 10.9-, and 4.7-kb clones shown on the right side of the restriction maps (Fig. 2) and extending left and beyond the 0.5-kb SmaI fragment. The restriction map of the maize *rrn18* gene, generated by computer analysis of the published sequence data of Chao et al. (1983, 1984) (Fig. 2, bottom), compared to the pearl millet map shows the similarity of the pearl millet and maize genes.

The *coxI* maize probe was hybridized to restriction digests of the four clones (Fig. 3C). The pUC vector was not removed from the blotted DNA or the probe, so the probe hybridized to pUC in each lane and is labeled pUC. The hybridization data below concerns only the fragments of the cloned inserts. In the 4.7-kb clone, the *coxI* probe hybridized to a 3.7-kb PstI-XhoI fragment and a 2.0-kb PstI-SmaI fragment. In the 10.9-kb clone, the *coxI* probe hybridized to a 4.9-kb XhoI fragment (PX lane), a 8.0-kb SmaI fragment (PS lane), and a 4.8-kb PstI-BamHI fragment. In the 13.6-kb clone, the *coxI* probe hybridized to 9.5-kb SmaI (PS lane), 7.4-kb XhoI (PX lane), and 6.0-kb PstI-BamHI fragments. The above fragments all overlap *coxI*, as indicated on the restriction map of Fig. 2. As with the *rrn18-rrn5* probe, the *coxI* probe did not hybridize to the 9.7-kb clone.

Transcriptional analyses

Transcription of the pearl millet clones was investigated in three ways: (1) by hybridizing Southern transfers of the cloned mtDNA fragments to end-labeled total mtRNA probes; (2) by using the pearl millet clones as probes to hybridize Northern blots of total mtRNA from cms, revertant, and normal cytoplasms; and (3) by hybridizing Northern blots with the maize *coxI* gene probe.

The end-labeled mtRNAs hybridized strongly to the same fragments of the 4.7-, 10.9-, and 13.6-kb clones that hybridized to the *rrn18-rrn5* maize gene probe. Those labeled mtRNAs also hybridized weakly to the fragments that hybridized to the *coxI* gene. Figure 4 shows the hybridization patterns of end-labeled mtRNA hybridized to the clones that were restricted with SmaI and XhoI (left), and the restriction patterns of the ethidium-bromide-stained gel (right). In the 4.7-kb clone, the RNA probe hybridized strongly to 1.2- and 3.7-kb (pUC plus a 1.0-kb fragment) fragments, weakly to a 2.0-kb fragment, and very weakly to a 0.5-kb fragment. In the 10.9-kb clone, the RNA probe hybridized strongly to a 1.2-kb fragment, moderately to the 1.0- and 2.3-kb fragments, weakly to a 3.3-kb fragment, and very weakly to a 0.5-kb fragment. In the 13.6-kb clone, the RNA probe hybridized strongly to 1.2- and 3.7-kb (pUC plus a 1.0-kb fragment) fragments and very weakly to 0.5-, 1.5-, and

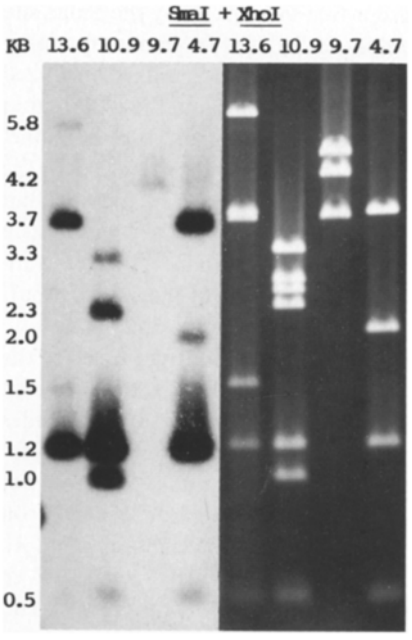

Fig. 4. Pearl millet clones digested with SmaI and XhoI hybridized to an end-labeled pearl millet total mtRNA probe (*left*) and the ethidium-bromide-stained gel (*right*). Sizes of hybridized fragments are given in kilobases on the *left*

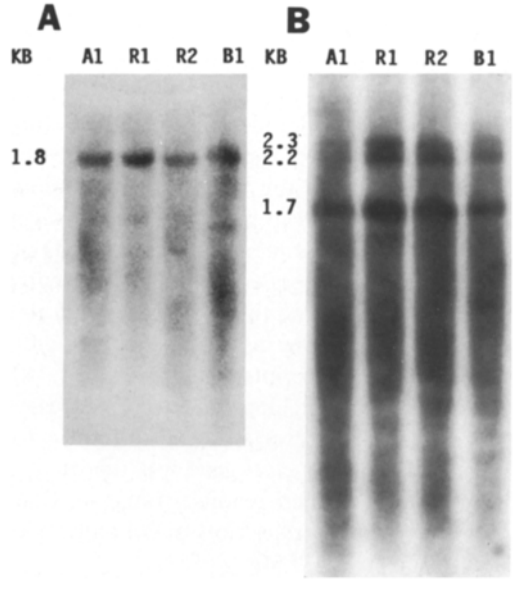

Fig. 5 A and B. Northern blots of total pearl millet mtRNA hybridized with the 4.7-kb clone (**A**) and the maize *coxI* gene (**B**), showing transcripts with homology to those probes. Sizes are given in kilobase (kb)

5.8-kb fragments. The three clones showed strong hybridization of the RNA probe to the fragments to the right of the 0.5-kb SmaI fragment (the *rrn18* region), and weak hybridization to the 0.5-kb SmaI fragment and fragments to the left of it (the *coxI* region) on the restriction maps (Fig. 2). The RNA probe also hybridized very weakly to a 4.2-kb fragment in the 9.7-kb clone, indicating that a portion of that fragment may be transcribed.

Northern blots of total mtRNA from the S-A1, revertant (R1 and R2), and normal fertile lines hybridized with the 4.7-, 10.9-, and 13.6-kb pearl millet probes. Each probe showed only a single transcript of 1.8 kb expressed uniformly across the cytoplasms. The hybridization pattern of the 4.7-kb clone used as the probe is shown in Fig. 5A. The 9.7-kb clone probe did not hybridize to the Northern blots. To better compare the *coxI* transcripts across the cms, revertant, and normal cytoplasms, Northern blots were hybridized to the maize *coxI* gene probe (Fig. 5B). That probe hybridized to transcripts of about 2.3, 2.2, and 1.7 kb, which were uniformly expressed across cytoplasms.

Discussion

mtDNA hybridization analyses

Maize mitochondrial gene probes hybridized readily to pearl millet mtDNA sequences under the relatively high stringency of this study, i.e., 0.3 × SSC at 65 °C for 15 min. At this stringency, it is presumed that these maize gene probes are hybridized to the pearl millet counterpart genes. That presumption is further supported by preliminary sequencing data of the pearl millet clones (unpublished results), showing that the pearl millet *coxI* and *rrn5* genes have a high degree of sequence homology with the maize genes. Probes containing the maize *coxI*, *rrn18*, and *rrn5* genes hybridized differentially to the Southern blots of the cms and fertile revertant mtDNAs (Fig. 1). The perfect correlation of fragment rearrangement (the loss of the 4.7-kb fragment and the gain of the 9.7-kb fragment) with reversion from cms to fertility in eight independent spontaneous revertants suggests that a gene or altered gene responsible for the expression of cms may be located on those rearranged fragments.

In Fig. 1, the hybridization of the *rrn18-rrn5* probe to the 13.6-kb fragment of the S-A1 (A1) and normal (B1) mtDNA appears to be relatively weaker than that of the 10.9- and 4.7-kb fragments of the S-A1, and the 10.9- and 10.0-kb fragments of the normal lines. That trend of weaker hybridization of the 13.6-kb fragment is not obvious in the hybridization patterns of the *coxI* probe (Fig. 1B), which argues against a reduced copy number of the 13.6-kb fragment being an explanation of the weaker hybridization. The cause and significance of that observation are unknown.

Clone mapping and hybridization

The identical patterns on the restriction maps shown in Fig. 2 verify that the cloned fragments contained repeated segments, i.e., the PstI-XhoI, XhoI-SstII, SstII-

SstII, SstII-SmaI, SmaI-SmaI, and SmaI-EcoRV fragments on the right of the 4.7-, 10.9-, and 13.6-kb clones and the left ends of the 13.6- and 9.7-kb clones. Since the maize *rrn18-rrn5* probe contained both ribosomal genes, the hybridization data did not answer the question of whether or not both pearl millet ribosomal genes were present. The similarity of the pearl millet restriction map to that of the maize *rrn18* gene is good evidence that the pearl millet *rrn18* gene is present. Preliminary sequencing data (unpublished results) indicated that the pearl millet *rrn5* gene is present and is closely linked to the *rrn18* gene as in maize.

Although the maize *coxI* probe contained flanking sequences, preliminary sequencing data (unpublished results) verified that the pearl millet coding sequences of *coxI* and *rrn5* are present on the hybridized pearl millet fragments, as indicated on the map in Fig. 2. The hybridization and preliminary sequencing data have also verified that the pearl millet *coxI* is closely linked to *rrn5* and that *rrn5* is closely linked to *rrn18* on each of the 4.7-, 10.9-, and 13.6-kb clones. At least three copies of the *rrn18* and *rrn5* genes were present in the male-sterile cytoplasm, S-A1. Upon reversion to fertility, two of the three *rrn18* and *rrn5* copies were lost (those on the 4.7- and 13.6-kb fragments), with the exception that one of eight revertants retained the 13.6-kb fragment. The male-sterile cytoplasm contained four copies of *coxI*, while two copies located on the 4.7- and 13.6-kb fragments were lost upon reversion. As with the ribosomal genes, one revertant retained the 13.6-kb fragment. The above data could fit the model presented by Fauron et al. (1990), where the cms-T genomic structure was found to contain four products of recombination due to an active repeat, and only two of those four products were found in the revertant. According to their model, reversion to fertility involved complicated intramolecular and intermolecular recombination events of two active repeats and some unknown mechanism that selectively eliminated or amplified recombination intermediates, resulting in a master chromosome with some sequences deleted and other sequences duplicated. In their case, the *urf13* gene was deleted; in our case, the *coxI*, *rrn5*, and *rrn18* genes located on the 4.7- and 13.6-kb fragments were deleted.

Transcriptional analyses

Transcripts that hybridized to the cloned pearl millet mtDNA and the maize genes were present in the isolated pearl millet mtRNA. End-labeled mtRNA probes hybridized to the same sequences of Southern blots of the three pearl millet clones, as did the maize *rrn18-rrn5* and *coxI* maize probes. In the Southern blots of Fig. 4, the 1.0-kb XhoI-PstI fragment of the 4.7- and 13.6-kb clones (right of Fig. 2 map) was attached to the pUC vector, giving a 3.7-kb fragment, while in the 10.9-kb clone that 1.0-kb fragment was cut free from pUC by the SmaI site of the pUC multiple cloning site. The fragments spanning the *rrn18* region (1.0- and 1.2-kb fragments) hybridized strongly, while those fragments spanning the *coxI* region (2.0 kb in the 4.7-kb clone, 3.3 kb in the 10.9-kb clone, and 5.8 kb in the 13.6-kb clone) hybridized weakly to the mtRNA probe. This could be explained by the fact that the ribosomal transcripts were much more abundant in the total RNA end-labeled probe than the *coxI* transcripts. The very weak hybridization of the 0.5-kb SmaI-SmaI fragment may be explained by its small size.

The end-labeled mtRNA probe also hybridized to the 2.3-kb SmaI-XhoI fragment of the 10.9-kb clone (labeled Z on Fig. 2), suggesting that an unidentified gene resides in region Z. Since the 10.9-kb fragment is in all cytoplasms, that undefined transcribed region is probably not important in cms. Very light hybridization occurred on the 1.5-kb fragment of the 13.6-kb clone and on a 4.2-kb fragment (pUC plus the 1.5-kb fragment) of the 9.7-kb clone, suggesting that an unidentified gene may extend onto those PstI-SmaI (left end) fragments.

Northern blots, hybridized with probes of each pearl millet clone, showed only one 1.8-kb transcript, although transcripts were expected for each of the *rrn18*, *rrn5*, and *coxI* genes. In addition, hybridization was uniform across the cms, revertant, and normal cytoplasms (Fig. 5A). As with the end-labeled mtRNA probes, we believe that the abundance of ribosomal mtRNA transcripts in the Northern blots obscured the signals of the *coxI* transcripts. Increased hybridization intensities (not shown) did not show the *coxI* transcripts.

To avoid interference of the ribosomal transcripts, the maize *coxI* gene was used to hybridize the Northern blots (Fig. 5B). Preliminary sequencing data verified that the pearl millet *coxI* coding sequences had a high degree of homology to maize *coxI* and that it would be suitable to use the maize gene as a probe instead of subcloning the pearl millet gene. Three transcripts of 2.3, 2.2, and 1.7 kb were noted on the Nothern blots and were expressed uniformly across the cms, revertant, and normal cytoplasms (Fig. 5B). The maize *coxI* gene was reported to consist of a continuous, open reading frame of 1,584 nucleotides, and two major transcripts of 2.4 and 2.3 kb were detected (Isaac et al. 1985).

In both maize and petunia, reversion from cms to fertility was characterized by the loss of a mtDNA fragment. In our pearl millet S-A1 system, reversion is always accompanied by the loss of the 4.7-kb PstI fragment. However, we were not able to detect differential transcription patterns among the cms and fertile revertants, as was done with both maize (Dewey et al. 1987) and petunia (Young and Hanson 1987). The lack of detectable transcript differences between cms and revertant cytoplasms neither supports nor refutes involvement of genes on the cloned segments in cms, but might indicate

that a mechanism may be operating in pearl millet that does not alter transcript length sufficiently to be detected by blot hybridization.

The 4.7-kb fragment is the site of the *coxI*, *rrn18*, and *rrn5* genes. Aberration in one or more of those genes may disturb the mitochondrial function sufficiently to produce the cms phenotype. Sequencing of the pearl millet *coxI* genes is underway to explore whether or not alterations occur in those genes that may have a role in cms determination.

References

Braun CJ, Levings CS III (1985) Nucleotide sequence of the F_1-ATPase alpha subunit gene from maize mitochondria. Plant Physiol 79:571–577

Chao S, Sederoff RR, Levings CS III (1983) Partial sequence analysis of the 5S and 18S rRNA region of the maize mitochondrial genome. Plant Physiol 71:190–193

Chao S, Sederoff RR, Levings CS III (1984) Nucleotide sequence and evolution of the 18S ribosomal RNA gene in maize mitochondria. Nucleic Acids Res 12:6629–6644

Chase CD, Pring DR (1986) Properties of the linear N1 and N2 plasmid-like DNAs from mitochondria of cytoplasm male-sterile *Sorghum bicolor*. Plant Mol Biol 6:53–64

Dale RMK, Mendu N, Ginsburg H, Kridl JC (1984) Sequence analysis of the maize mitochondrial 26S rRNA gene and flanking regions. Plasmid 11:141–150

Dewey RE, Levings CS III, Timothy DH (1985a) Nucleotide sequence of ATPase subunit 6 gene of maize mitochondria. Plant Physiol 79:914–919

Dewey RE, Schuster AM, Levings CS III, Timothy DH (1985b) Nucleotide sequence of F_0-ATPase proteolipid (subunit 9) gene of maize mitochondria. Proc Natl Acad Sci USA 82:1015–1019

Dewey RE, Levings CS III, Timothy DH (1986) Novel recombinations in the maize mitochondrial genome produce a unique transcriptional unit in the Texas male-sterile cytoplasm. Cell 44:439–449

Dewey RE, Timothy DH, Levings CS III (1987) A mitochondrial protein associated with cytoplasmic male sterility in the T cytoplasm of maize. Proc Natl Acad Sci USA 84:5374–5378

Dewey RE, Siedow JN, Timothy DH, Levings CS III (1988) A 13-kilodalton maize mitochondrial protein in *E. coli* confers sensitivity to *Bipolaris maydis* toxin. Science 239:293–295

Fauron CMR, Havlik M, Brettell RIS (1990) The mitochondrial genome organization of a maize fertile cms-T revertant line is generated through recombination between two sets of repeats. Genetics 124:423–428

Feinberg AP, Vogelstein B (1983) A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 137:266–267

Forde BG, Leaver CJ (1980) Nuclear and cytoplasmic genes controlling synthesis of variant mitochondrial polypeptides in male-sterile maize. Proc Natl Acad Sci USA 77:418–422

Fox TD, Leaver CJ (1981) The *Zea mays* mitochondrial gene coding cytochrome oxidase subunit II has an intervening sequence and does not contain TGA codons. Cell 26:315–323

Hanson MR, Conde MF (1985) Functioning and variation of cytoplasmic genomes: lessons from cytoplasmic-nuclear interactions conferring male sterilities in plants. Int Rev Cytol 94:213–267

Holmes DS, Quigley M (1981) A rapid boiling method for preparation of bacterial plasmids. Anal Biochem 114:193–197

Isaac PG, Jones VP, Leaver CJ (1985) The maize cytochrome *c* oxidase subunit I gene: sequence expression and rearrangement in cytoplasmic male plants. EMBO J 4:1617–1623

Maniatis T, Fritsch EF, Sambrook J (1982) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor /NY

Pearson OH (1981) Nature and mechanisms of cytoplasmic male sterility in plants: a review. Hort Science 16:482–487

Schaffer HE, Sederoff RR (1981) Improved estimation of DNA fragment lengths from agarose gels. Anal Biochem 115:113–122

Smith RL, Chowdhury MKU (1989) Mitochondrial DNA polymorphism in male-sterile and -fertile cytoplasms of pearl millet. Crop Sci 29:809–814

Smith RL, Chowdhury MKU, Pring DR (1987) Mitochondrial DNA rearrangements in *Pennisetum* associated with reversion from cytoplasmic male sterility to fertility. Plant Mol Biol 9:277–286

Wise RP, Pring DR, Gengenbach BG (1987) Mutation to male fertility and toxin insensitivity in Texas (T)-cytoplasm in maize is associated with a frame shift in a mitochondrial open reading frame. Proc Natl Acad Sci USA 84:2858–2862

Young EG, Hanson M (1987) A fused mitochondrial gene associated with cytoplasmic male sterility is developmentally regulated. Cell 50:41–49