COMPARATIVE MOLECULAR ANALYSIS OF MITOCHONDRIAL GENOME IN TWO CYTOPLASMIC MALE STERILE SYSTEMS OF COTTON

Jinfa Zhang, G. Mara-Koosham, and Yingzhi Lu
Department of Agronomy and Horticulture
New Mexico State University
Las Cruces, NM
J. McD. Stewart
Department of Crop, Soil, and Environmental Sciences
University of Arkansas
Fayetteville, AR

Abstract

A new cytoplasmic male sterility (CMS) and restoration system denoted as CMS-D8 and D8 restorer, which was developed by transferring cytoplasm and restorer gene from the American diploid wild species, *Gossypium trilobum* Skov. (D_8 genome) into cultivated tetraploid cottons (*G. hirsutum* L. and *G. barbadense* L.), can be utilized for economic hybrid cotton seed production. Two independent dominant restorer genes, Rf_1 from the D2 restorer line transferred from *G. harknessii* Brand (D_2 genome) and Rf_2 from the D8 restorer line were identified. The objectives of the present study were, (1) to test the efficiency of a set of MtDNA universal primers in cotton; and (2) to identify potential MtDNA markers that can be used to differentiate CMS-D2 and CMS-D8 cytoplasms in comparison with upland cotton normal fertile cytoplasm. Of 36 primer pairs designed for amplifying introns, intergenic regions, or genes on cotton MtDNA genome, 16 pairs produced amplifications with expected fragment sizes based on *Arabidopsis thaliana* MtDNA genome sequence, with the exception of nad7 and rps12. Our work using MtDNA universal primers revealed no STS polymorphism among the three cytoplasms (AD1, D2 and D8), confirming that MtDNA genes in cotton are highly conserved in sequences as in other plant species. DNA sequencing on the amplified products could identify SNPs in the CMS cytoplasms in comparison with the normal fertile AD1 cytoplasm.

Introduction

The main function of mitochondria in higher plants is the production of ATP and NADH via oxidative phosphoylation to supply the major cellular energy needs. Therefore, any disruption in the respiratory chain (Figure 1) potentially could be very detrimental.

Plant mitochondrial (Mt) genomes, varying in size ranging from 200 kb to 2400 kb, contain 50-60 genes including 30-35 protein-encoding genes based on the complete MtDNA genome sequences from rice, *Arabidopsis*, *Brassica napus*, and sugar beet (Figure 2, Kubo et al., 2000). Except for genes encoding for ribosomal proteins, these protein genes encode proteins for complex I (NADH dehydrogenases, nad1 to 9), II (succinate dehydrogenases, sdhB, C, and D), III (cytochrome b, cob), IV (cytochrome c oxidases, coxI, II, and III), V (ATP synthases, atp1, 4, 6, 8, and 9), and cytochrome c biogenesis (4-6 ccb genes).

Cytoplamsic male sterility (CMS), an inability to produce functional pollen found in more than 150 plant species, is a particular mitochondrial dysfunction associated with a mutation of the mtDNA genome. In several plant species, CMS-associated genes have been identified that are often chimeric open reading frames (ORFs) involving the rearrangements of functional Mt genes, such as atp1, atp6, atp9, coxI and coxII, etc. (Schnable and Wise, 1998).

Two CMS systems with cytoplasms introduced from American wild species, *G. harknessii* (D2) and *G. trilobum* (D8) into cotton, have been reported (Meyer, 1975; Stewart, 1992). Our previous RFLP analysis indicated that the CMS-D2 MtDNA is different from the normal tetraploid cytoplasm in coxII and atp1 genes (Feng et al., 2000). However, no information on the CMS-D8 MtDNA has been reported. The objectives were, (1) to test the efficiency of a set of MtDNA universal primers in cotton; and (2) to identify potential MtDNA markers that can be used to differentiate CMS-D2 and CMS-D8 cytoplasms in comparison with upland cotton normal fertile cytoplasm.

Materials and Methods

Plant Materials

(1) D2 restorer lines (D2R) containing CMS-D2 cytoplasm and restorer gene Rf1: B411R and B418R. (2) Two pairs of CMS-D8 and its maintainer lines: CMS-D8 8518 vs. 8518 and CMS-D8 ST474 vs. ST474. (3) D8 restorer lines (D8R) containing CMS-D8 cytoplasm and restorer gene Rf2: D8R 8518 and D8R ST474. These materials were grown in the field, Las Cruces, NM, 2003.

DNA Isolation
Mini-prep method (Zhang and Stewart, 2000)

PCR and Electrophoresis
A set of 40 PCR primer pairs specific for plant MtDNA were collected or designed for amplifying introns, intergenic regions, or genes on cotton MtDNA genome. PCR amplification conditions were, 1 cycle of 4 min at 94C, followed by 40 cycles of 15 s at 94C, 30s at 53C and 30 s at 72C and a final 10 min extension at 72C. The PCR products were separated by electrophoresis through 1.4% agarose gel.

Results

Of 36 primer pairs tested, 16 pairs produced amplifications with expected fragment sizes based on *Arabidopsis thaliana* MtDNA genome sequence, with the exception of nad7 and rps12 (Table 1).

ATP synthase genes (atp) in complex V: Of 4 pairs of primers for atp1, 4, 6, and 9, only the primer pair for atp4 amplified a fragment with an expected size of 0.4 kb (Figure 3). However, no polymorphism was detected among CMS-D2, CMS-D8 and AD1 mtDNA.

Cytochrome c oxidase genes (cox) in Complex VI: Of 6 pairs of primers for coxI, II, and III, 5 pairs (2 for coxI, 2 for coxII, and 1 for coxIII) produced PCR products of anticipated sizes. Again, no polymorphism was seen (Figure 3).

NADH dehydrogenase genes (nad) in Complex I: Of 16 pairs of primers for nad genes, 4 (for nad1, 6, 7, and 9) produced amplified fragments of expected sizes. Unfortunately, no polymorphism was revealed (Figure 3)

Ribosomal protein genes (rps and rpl): There are about 10-13 genes. Of 5 primer pairs, 4 showed PCR amplification. Again, no size differences were detected (Figure 3).

Ribosomal RNA genes (rrn): Of 3 rrn Mt genes, rrn5 was chosen for the study using 2 pairs of primers. 1 pair of primers showed strong amplification of expected size (0.3 kb). No size polymorphism among the three cytoplasms was seen (Figure 3)

Cytochrome c biogenesis genes (ccb): 1 pair of primers was tested, and again no polymorphism was detected (Figure 3).

Conclusions and Future Directions

1. Our work using universal primers for MtDNA revealed no STS polymorphism among the three cytoplasms, confirming that MtDNA genes in cotton, as in other plant species are highly conserved in sequences.
2. PCR conditions will need to be adjusted for optimum amplification of each primer pairs.
3. MtDNA specific PCR products will be digested using frequent (4-bp) restriction enzymes to reveal PCR-RFLP.
4. The amplified PCR products will be sequenced to determine if SNPs are present among the cytoplasms
5. MtDNA gene expression will be compared.

References

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Table 1. Primer pairs that amplified MtDNA in cotton.

MtDNA gene	Primer pair	Size amplified in cotton (kb)	Size from *Arabidopsis* (bp)
atp4 (orf25)	ORF25F:ORF25R	0.5	504
coxI	COX1F:COX1R	1.4	1360
coxI	COX Ia-F:COX1a-R	0.8	?
coxII	COX2/1F:COX1R	0.4	376
coxII	COX2/2F:COX2/2R	1.5	1421
coxIII	COX3F:COX3R	0.7	704
nad1	NAD1/2F:NAD1/2R	1.0	1084
nad6	NAD6F:DAD6R	0.6	605
nad7	NAD7/4F:NAD7/4R	1.5	1952
nad9	NAD9F:NAD9R	0.5	491
rpl5	RPL5F:RPL5R	0.4	432
rps3	RPS3F:RPS3R	>1.5	2076
rps4	RPS4F:RPS4R	0.9	950
rps12	RPS12F:NAD3-2R	0.4	700
rrn5	RRN5F:RRN18-1R	0.3	273
ccb203	CCB203F:CCB203R	0.5	511

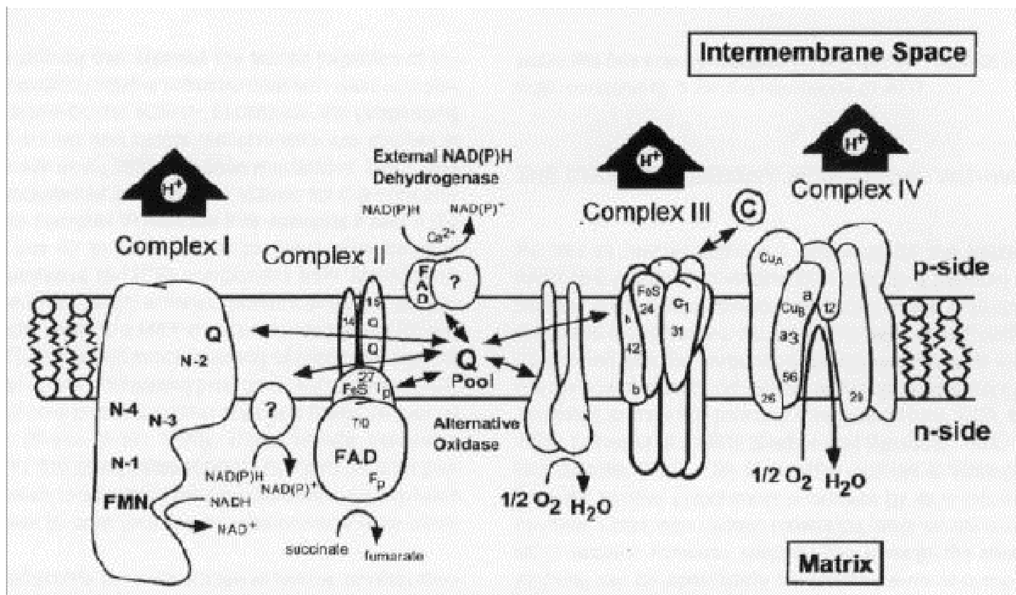


Figure 1. Respiratory chain in higher plants.

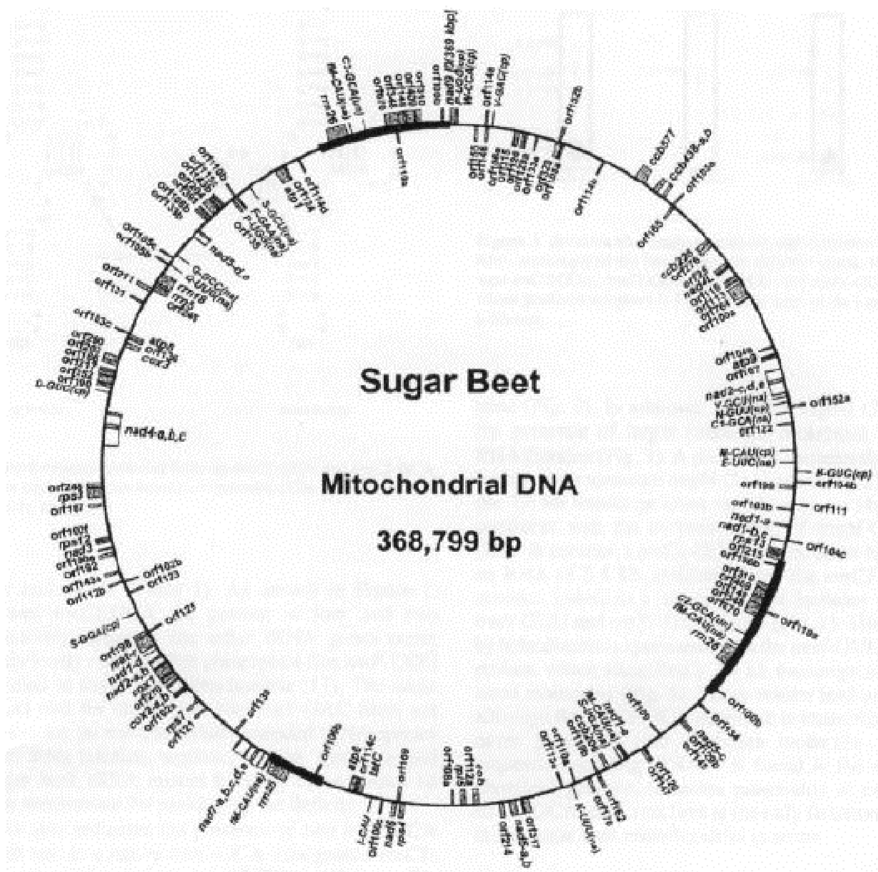
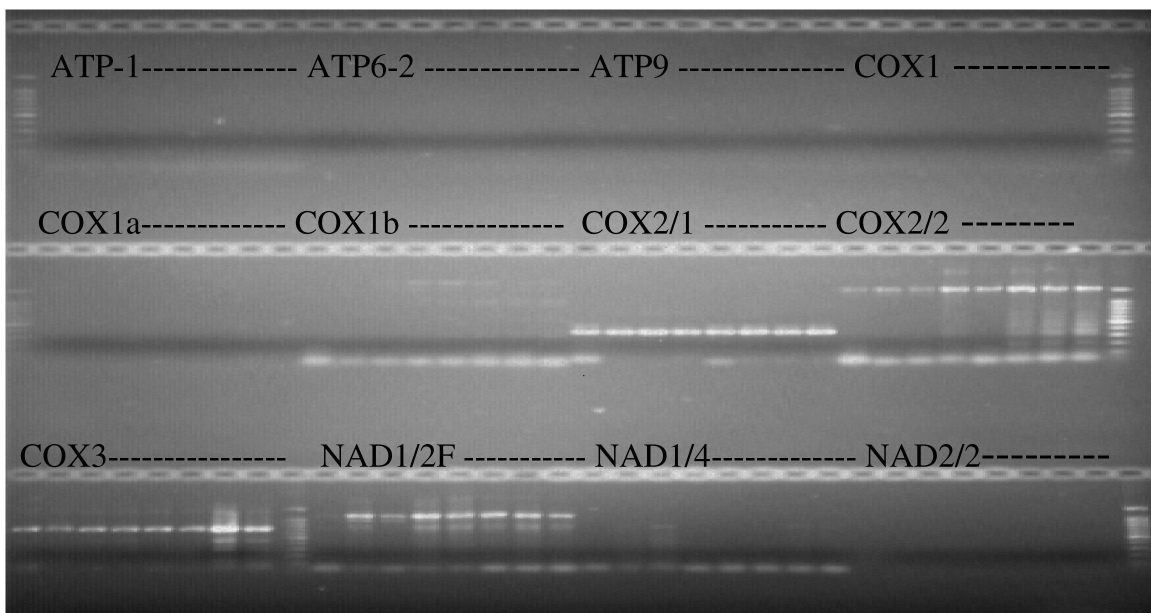
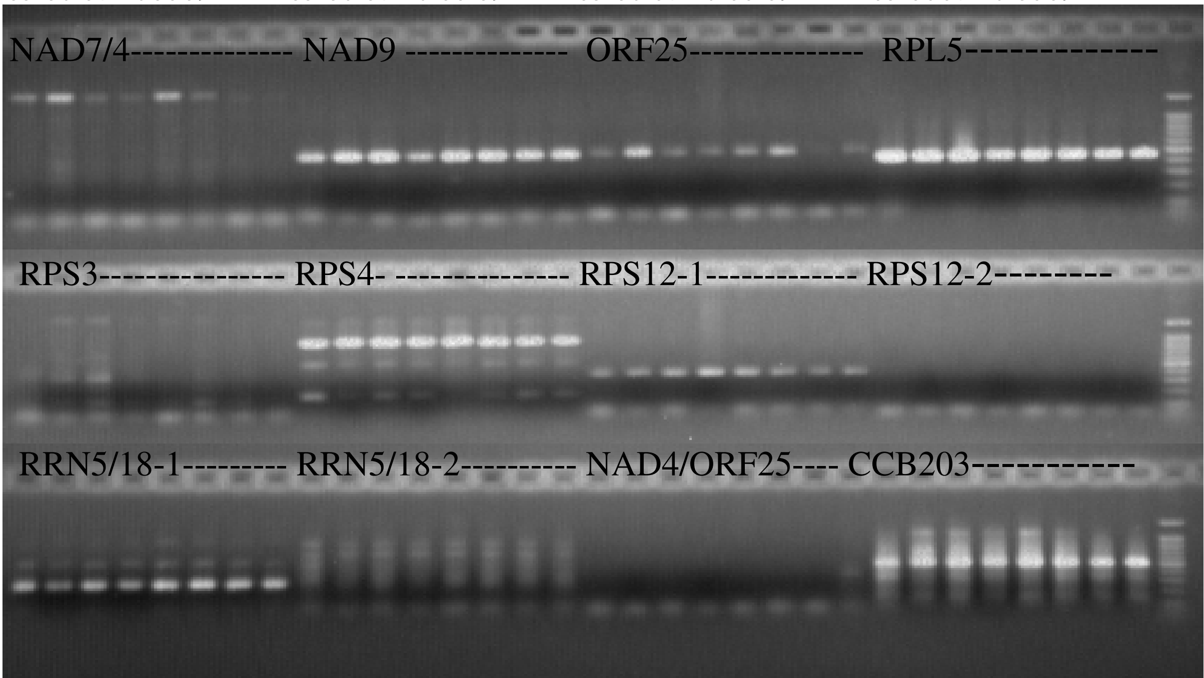
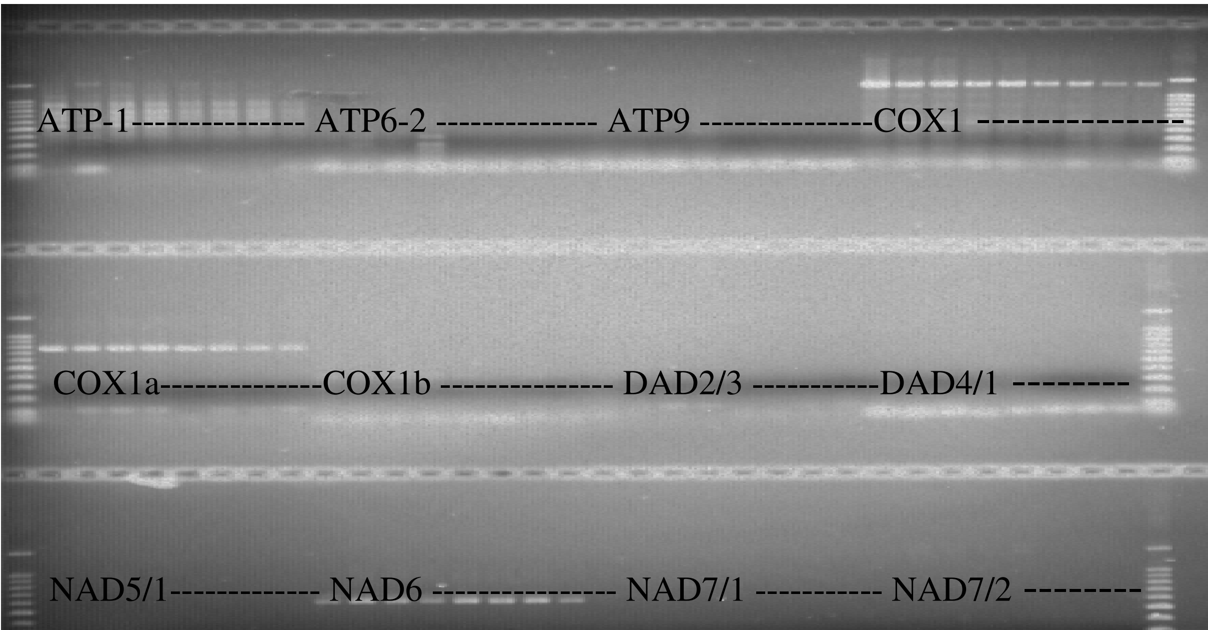


Figure 2. MtDNA structure and physical map.







35: D2R-B411R
36: D2R-B418R
37: 8518
40: ST474
38: CMS-D8 8518
39: D8R 8518
41: CMS-D8 ST474
42: D8R ST474

Figure 3. PCR amplification in cotton using MtDNA universal primers.