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Mitochondrial ATP synthase genes may be implicated in cytoplasmic male sterility in *Sorghum bicolor**

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Abstract. Incompatible nuclear-cytoplasmic interactions are responsible for the phenomenon of cytoplasmic male sterility in plants. We have analysed male sterile (2077A, 296A), maintainer fertile (2077B, 296B) and fertility restored (2077R, 296R) lines of sorghum for the restriction fragment locations of various mitochondrial genes and their transcripts. We report here a polymorphism in genes related to the ATP synthase complex between two different cytoplasms from the A and B set of lines of 2077 and 296. There is also a difference in the transcript size of the *atpA* gene between the A and B cytoplasms. We propose that incompatibility in nuclear cytoplasmic interactions may be explained in terms of incompatible subunits being synthesized by the mitochondria and nucleus for a multisubunit complex of the mitochondrial membrane such as ATPase.

Keywords. Cytoplasmic male sterility (CMS) mitochondria; ATP synthase; *atp*A gene.

1. Introduction

Hybrid seed production is facilitated by the development of cytoplasmic male sterile, maintainer and restorer lines. The male sterile and maintainer (fertile) lines have an identical nuclear background but different cytoplasms. Since sterility is maternally inherited, one possibility is that the mitochondrial genome is responsible for this trait. The involvement of the chloroplast genome has been ruled out by several studies (Belliard *et al* 1978; Clark *et al* 1985; Hanson 1991). Since the identical nuclear background causes sterility in one line but not in the other (the maintainer) it is clear that a given nuclear background is compatible with one cytoplasm but incompatible with the other. Apparently the sterility is related to the interaction of the nuclear genome with the mitochondrial genome (Bailley-Serres *et al* 1986b; Hakansson and Glimelius 1991).

Indian plant breeders have evolved several cytoplasmic male sterile (CMS) lines of sorghum in the past few decades and the molecular characterization of these lines is important to find out the differences between the sterile and fertile cytoplasms in a particular nuclear background. Further, the ability of the restorer line to restore fertility in the sterile cytoplasm raises the question of the nature of restorer genes at the molecular level. To address these questions a systematic study of existing CMS lines, their maintainers and restored lines is essential.

As a first step in this direction we have attempted to characterize the mitochondrial genome diversity of the male sterile (A), male fertile (B) and the restored line (R)

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belonging to 2077 and 296 lines of sorghum. In this communication we provide information on the location of several mitochondrial genes in these two sets of lines and the study of transcript sizes. The results indicate major polymorphic differences in the location of those genes that are associated with the assembly of the ATPase complex and suggest that the *atp*A gene product (α subunit) may be one of the polypeptides involved in male sterility.

2. Materials and methods

2.1 *Seed material*

Seeds of following lines were used in this study: 2077A, 296A (CMS lines), 2077B, 296B (maintainer fertile lines) and 2077R, 296R (restored fertile lines). These lines have been developed by Indian plant breeders.and basically contain a Kafir nucleus and Milo cytoplasm. The seeds were obtained from Prof. A R Dabholkar of the All India Co-ordinated Sorghum Improvement Project, Indore.

2.2 *Mitochondrial gene probes*

The gene probes used in this study were *cox*I, *cox*II, *atp*6 and *atp*9 from maize (provided by Dr C S Levings III, North Carolina State University) and *cob* and *atp*A from maize (provided by Dr C J Leaver, University of Oxford, UK). In most cases, fragments internal to the gene were prepared from the plasmid by digesting with appropriate restriction endonucleases. Radiolabelled probes were prepared by incorporating [α-^{32}P]dATP through nick translation or random priming (Sambrook *et al* 1989).

2.3 *Mitochondrial DNA isolation and Southern transfer*

Mitochondrial DNA was isolated from seven day old etiolated seedlings basically by a procedure as described by Chase and Pring (1986) with some modification (Nath *et al* 1993). To ensure plasmid DNA recovery mitochondria were lysed in the presence of high amount of proteinase-K (200 μg/ml) in the lysis buffer. After the first precipitation, the DNA was dissolved in a solution containing 6·5 M urea and 1 M NaCl. The solution was heated at 65°C for 10 min, cooled and the DNA was reprecipitated by the addition of 2 volumes of chilled ethanol. After pelleting the precipitated DNA, it was dissolved in TE buffer (Tris. Cl 10 mM, EDTA 1 mM, pH 8.0) and once again precipitated with ethanol, in the presence of 0·3 M sodium acetate, for about 1-2 h. This was then pelleted by centrifugation, dissolved in TE and used for restriction digestion. Purified DNA (3-5 μg) was digested with the restriction endonucleases *Bam*HI, *Hind*III, *Sma*I, *Cla*I, *Dra*I, *Sac*II and *Pvu*II (all obtained from Stratagene) for 4-5 h under the conditions as described in the manufacturer's protocol. Digested DNA was resolved by submarine gel electrophoresis in 0·8% agarose gel in TAE (Tris-acetate 40 mM, EDTA 1 mM, pH 8·0) electrophoresis buffer, at 4V/cm, stained in ethidium bromide (1 μg/ml) and photographed. The restricted fragments were transferred on to a nylon membrane (Nytran, S and S) by capillary transfer as described by Southern (1975) and cross-linked by UV.

2.4 *Isolation of mtRNA and northern transfer*

RNA was prepared from the mitochondria of etiolated seedlings as described earlier (MacDonald *et al* 1987). Northern transfer of total mtRNA was achieved by electrophoresing 20 µg of total mtRNA in 1% denaturing formaldehyde agarose gel followed by capillary transfer as described by Sambrook *et al* (1989).

2.5 *Hybridization*

Hybridizations of Southern and northern blots were carried out at 42°C in the hybridization mixture consisting of 50% formamide, 0·9 M NaCl, 0·2 M Tris. Cl (pH 8·0), 5 × Denhardt's solution, 1% SDS and sonicated salmon sperm DNA (100 µg/ml). The hybridization was carried out for 16-18 h after the addition of denatured α-^{32}P-labelled DNA probes (1 × 10^6dpm/ml hybridization mixture). The blots were washed in 2 × SSC (NaCl 0·3 M, sodium citrate 30 mM), 0·1 % SDS for 5 min followed by 0·5 × SSC, 0·1% SDS for 15 min twice and finally in 0·1 × SSC, 0·1% SDS for 5 min. Kodak X-0 mat XK-5 film was exposed to the blots for 1-2 days depending upon signal intensity and autoradiogram developed.

3. Results

3.1 *Comparison of 2077A, 2077B and restored* (*R*) *lines*

The mitochondrial membrane consists of several protein complexes that participate in membrane associated electron transport and synthesis of ATP. Of these complexes mitochondrial genome encodes for some of the polypeptides whereas the others are imported from the nucleus. Using DNA probes derived from the mitochondrial genome of maize the locations of some of the mitochondrial genes on the DNA restriction fragments has been determined by Southern analysis. Figure 1 shows

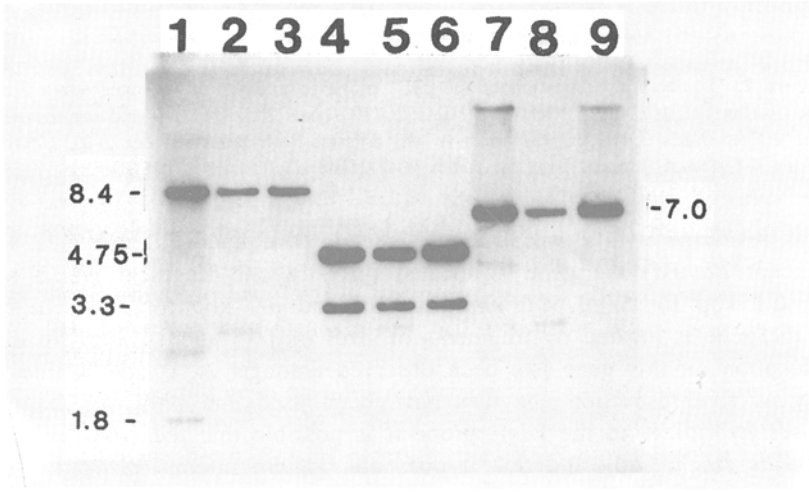

Figure 1. Southern hybridization of the *cox*l probe to mtDNA of the male sterile line 2077A (lanes 1,4,7), maintainer line 2077B (lanes 2,5,8) and the restored line 2077R (lanes 3, 6, 9). DNA was digested with *Bam*HI (lanes 1-3), *Hind*III (lanes 4-6) and *Sma*I (lanes 7-9).

the location of the *cox*I gene (the gene responsible for the synthesis of subunit I of cytochrome oxidase) in the male sterile (2077 A), the maintainer fertile (2077 B) and the restored (R) line mtDNA restricted with three endonucleases *viz. Bam*HI, *Hind*III and *Sma*I. In each case an identical pattern was generated in all the three lines. Whereas *Bam*HI primarily yields a 8·4 kbp fragment possessing the *cox*I gene, *Hind*III yields two fragments of sizes 4·75 and 3·3 kbp. *Sma*I digestion yields primarily one band of about 7 kbp (intensity differences, both in this and subsequent figures, are due to variations in the amounts of DNA loaded). Since two of the restriction enzymes give only one band it appears that there is only one copy of the *cox*I gene unless one assumes that more than one copy is present and that both are in tandem without any site for *Bam*HI or *Sma*I between them. Since *Hind*III produces two fragments of somewhat different intensities—the 4·75 kbp being more intense than the 3 kbp it is likely that the single copy gene has a site for *Hind*III within it.

Figure 2 provides information on the location of *cox*II gene (encoding for subunit II of the cytochrome oxidase complex) when the mitochondrial DNA of the three

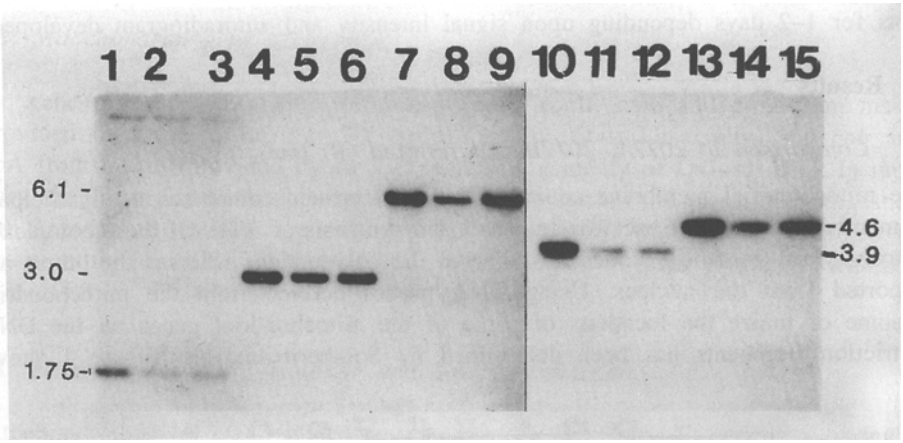

Figure 2. Souther hybridization of the *cox*II probe to mtDNA of the male sterile line 2077A (lanes 1,4,7, 12, 15), maintainer line 2077B (lanes 2, 5, 8, 11, 14) and the restored line 2077R (lanes 3, 6, 9, 10, 13). mtDNA was digested with *Bam*HI (lanes 1-3), *Hind*III (lanes 4-6), *Sma*I (lanes 7-9), *Dra*I (lanes 10-12) and *Cla*I (lanes 13-15).

lines was restricted with *Bam*HI, *Hind*III, *Sma*I, *Dra*I and *Cla*I enzymes. In each case (except *Bam*HI) the gene is located on only one fragment *viz.* 3 kbp for *Hind*III, 6·1 kbp for *Sma*I, 4·6 kbp for *Cla*I and 3·9 kbp for *Dra*I. In case of *Bam*HI the gene is located on fragments of sizes of 1·75 and 14 kbp. No diversity in the location on this gene has been observed amongst A, B and R lines. Since as many as five restriction enzymes have been used and only one fragment in most cases hybridizes to the *cox*II probe it is possible that the *cox*II gene is also present as a single copy and that it does not contain internal restriction site for any of the enzymes used here.

The restriction fragment location of the *cob* gene responsible for the synthesis of apocytochrome b is shown in figure 3. When restricted with four different

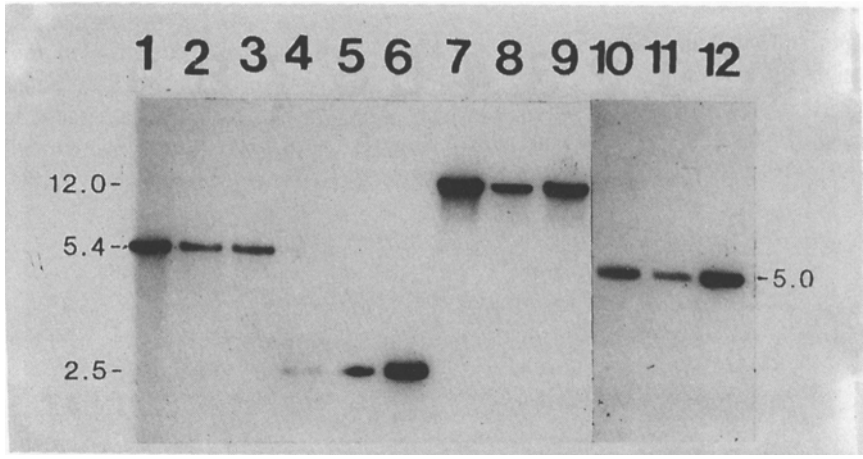

Figure 3. Southern hybridization of the *cob* probe to mtDNA of the male sterile line 2077A (lanes 1,4,7,10), maintainer line 2077B (lanes 2,5,8,11) and the restored line 2077R (lanes 3, 6, 9, 12). mtDNA was digested with *Bam*HI (lanes 1-3), *HindIII* (lanes4-6), *Sma*I (lanes 7-9) and *Cla*I (lanes 10-12).

enzymes namely *Bam*HI, *Hind*III, *Sma*I and *Cla*I, each lane lights up only one fragment on hybridization of the sizes 54, 25, 12 and 5 kbp respectively. The fragment size possessing this gene is identical in each case. Thus, there are no differences in the mitochondrial genomes of the three lines in terms of where this gene is located. Once again, the interesting possibility is that all the mitochondrial genomes tested possess only one copy of this gene and that the gene does not have a restriction site for these enzymes within it.

The ATP synthase complex of plant mitochondria is a multisubunit enzyme. Of the several subunits, the mitochondrial genome is known to encode subunit 6 and subunit 9. These are polypeptides of the membrane-bound hydrophobic F_0 component. The mitochondrial genome also encodes subunit A, which is one of the polypeptides of the F_1 component. Figure 4 shows the pattern generated for the *atp*6 gene on a Southern blot. The diversity in the location of this gene in the three lines when the DNA was restricted with *Bam*HI, *Hind*III and *Sma*I is apparent. In each case the pattern for the sterile (A) and the restored (R) line is identical whereas the fertile maintainer (B) line differs from the other two. In *Bam*HI restricted DNA the gene is located on two fragments, one with an approximate size of 6·8 kbp and another of size 5·0 kbp, in both A and R lines. The B line, however, shows only one band which is the 5 kbp band observed for A and R lines. In case of *Hind*III also the A and R lines show two bands around 3·0 and 1·9 kbp whereas the B line shows only one band at 1·9 kbp. Restriction by *Sma*I generates a pattern that shows two equally intense bands at 6·1 and 7 kbp for A and R lines but only one band at 7 kbp for B line.

The location of the *atp*9 gene on mitochondrial fragments as revealed by Southern analysis also shows that the A and R lines yield identical patterns whereas the B line is different (figure 5). A *Bam*HI digest of the mtDNA of the three lines shows a single band of about 14 kbp in all lines. In case of the *Hind*III digest, bands of 3·1, 3·4 and 4·2 kbp are seen in the A and R lines. The 4·2 kbp band is, however, missing in the B line. Similarly the *Sma*I digest shows bands of around 14·5 and 6·3 kbp common to all lines besides an additional fragment of 11·2 kbp

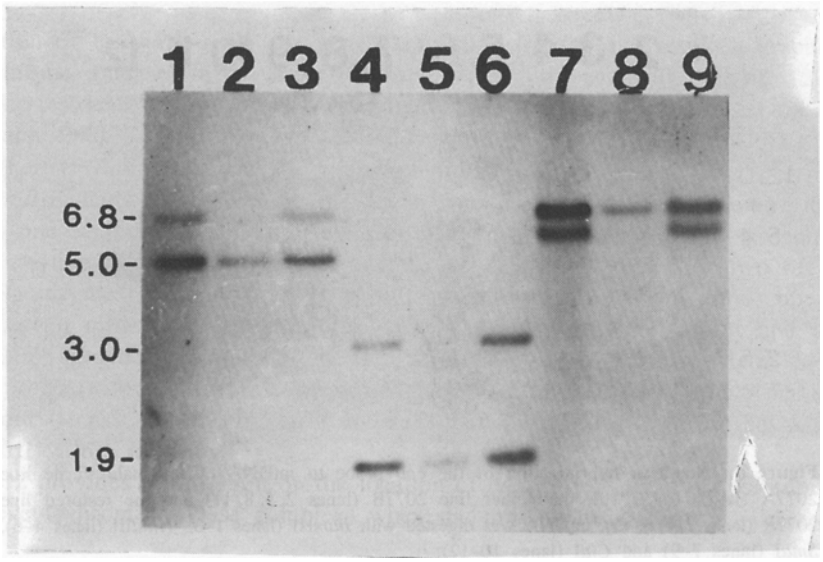

Figure 4. Southern hybridization of the *atp*6 probe to mtDNA of the male sterile line 2077A (lanes 1,4,7), rnaintainer line 2077B (lanes 2,5,8) and the restored line 2077R (lanes 3, 6, 9). mtDNA was restricted with *Bam*HI (lanes 1-3), *Hind*III (lanes 4-6) and *Sma*I (lanes 7-9)

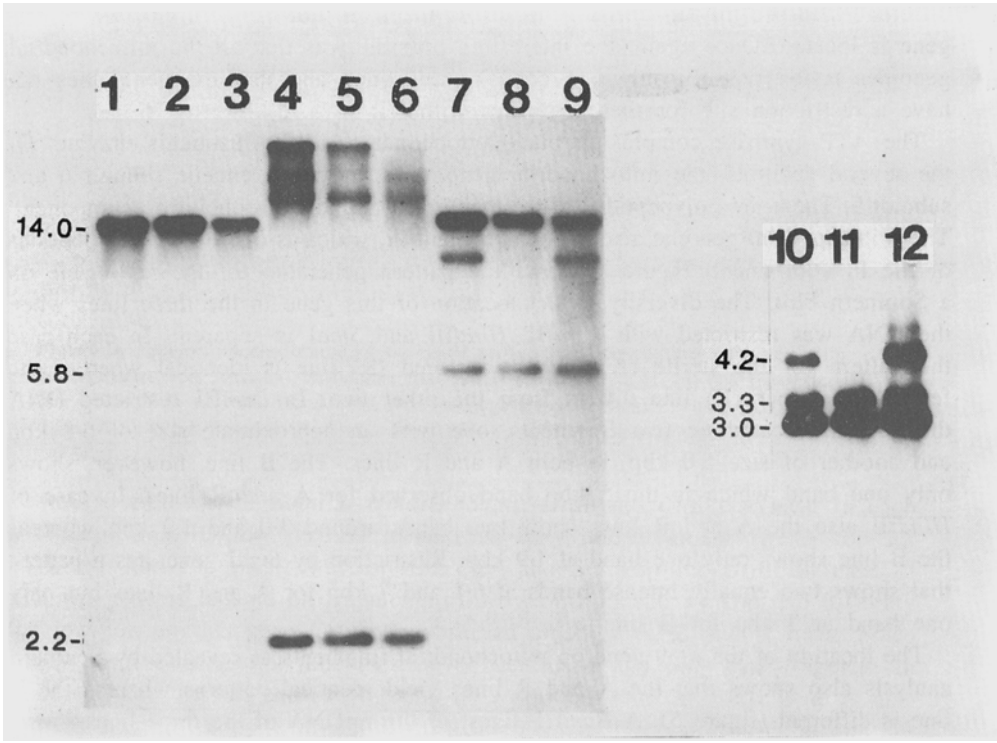

Figure 5. Southern hybridization of the *atp*9 probe to mtDNA of the male sterile line 2077A (lanes 1,4,7,10), maintainer line 2077B (lanes 2,5,8,11) and the restored line 2077R (lanes 3,6, 9, 12). mtDNA was restricted with *Bam*HI (lanes 1-3), *Pvu*II (lanes 4-6), *Sma*I (lanes 7-9) and *Hind*III (lanes 10-12).

present only in the A and R lines. Since the *atp*9 gene is only 240 bp long, the presence of 2 or more bands in some of the A and R digests suggest the existence of at least two copies of this gene.

The pattern relating to the *atp*A gene shown in figure 6 provides evidence for the existence of greater diversity between A and B lines. The A and R lines

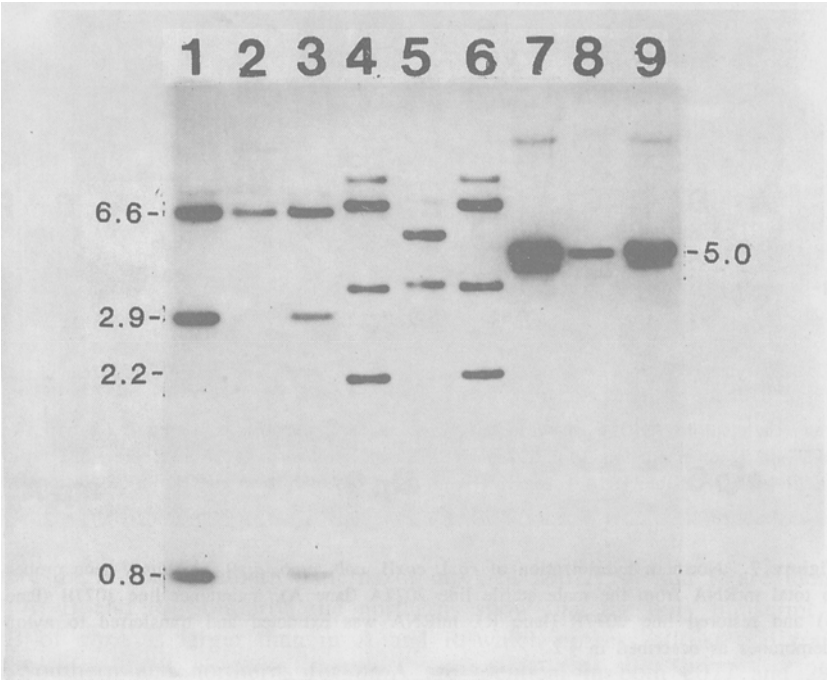

Figure 6. Southern hybrization of the *atp*A probe to mtDNA of the male sterile line 2077A (lanes 1,4.7), maintainer line 2077B (lanes 2,5,8) and the restored line 2077R (lanes 3, 6, 9). mtDNA was digested with *Bam*HI (lanes 1-3), *Hind*III (lanes 4–6) and *Sma*I (lanes 7–9).

generate identical patterns irrespective of the enzyme used for restriction analysis. A *Bam*HI and *Sma*I digest shows prominent bands of 6·6 and 5·0 kbp respectively which are common to all three lines. However, additional bands of 2·9 and 0·8 kbp for *Bam*HI and 4·5 kbp for *Sma*I can be seen only in the A and R lines. With *HindIII* the patterns of A and B lines do not match at all. Prominent bands of 7, 3·6 and 2·2 kbp can be seen in the A and R lines whereas the B line shows bands of 5.5 and 3·8 kbp.

Southern analysis indicates the location of a gene on a particular DNA fragment. The differences noted above could also be due to the difference in the presence of restriction sites in flanking regions without any difference in the gene itself, or to the presence of more than one copy of the gene in question. Major differences in the size of the coding portion of the gene can be determined by an examination of the transcripts through a northern analysis. Figure 7 shows the results when RNA gels were hybridized after electrophoresis with DNA probes for *cox*I, *cox*II, *cob*, *atp*6, *atp*9 and *atp*A. The patterns generated are more or less similar in all the three lines. The *atp*9 shows only one transcript of 0·62 kb whereas *cox*I gives

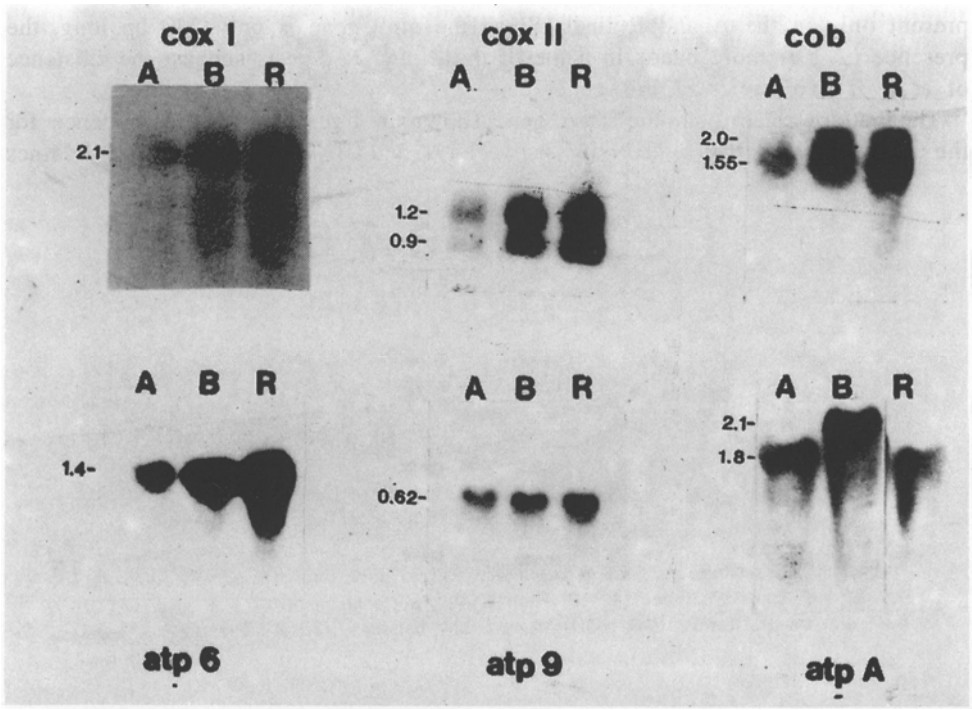

Figure 7. Northern hybridization of *cox*I, *cox*II, *cob*, *atp*6, *atp*9, and *atp*A gene probes to total mtRNA from the male sterile line 2077A (lane A), maintainer line 2077B (lane B) and restored line 2077R (lane R). mtRNA was extracted and transferred to nylon membranes as described in § 2.

a transcript of 2·1 kb. (We note that the coding sequence of *atp*9 gene is estimated to be 240 bp in size; the larger transcript could be derived from untranslated sequences.) There are two transcripts of 1·55 and 2·0 kb for *cob* and two transcripts of 0·9 and 1·2 kb for *cox*I. In case of *atp*6, only one transcript of 1·4 kb is observed. There are no differences in the transcript patterns between A, B and R lines. However, the northern analysis for *atp*A shows interesting differences. The A and R lines show two transcripts of 1·8 and 1·5 kb (seen only on long exposures) whereas the B line shows a somewhat larger single transcript of 2·1 kb. Thus, it appears that there may be major differences in the *atp*A gene and its transcript.

3.2 *Comparison of 296 A, B and restored line*

The 296 A (male sterile) is restored by a line (CS3541) which also restores 2077 A. One might therefore, expect 296 A and 2077 A to resemble each other at least in those respects which relate to male sterility. We have investigated gene location patterns in 296 A, B and its restored line.

The line 296 A possesses a cytoplasm (mitochondria) which is similar but not identical to 2077 A. This is brought out by the fact that 296 A possesses a number of plasmids totally absent in case of 2077 (data to be published separately). The

gene location patterns are also different from 2077 A. The gene location patterns in 296 A, B and R in respect of *cox*II, *cob* and *atp*6 are almost identical to those for 2077 A, B and R respectively (data not shown). On the other hand the *cox*I pattern generated by *Hind*III digestion for 296 lines is different from 2077 A, B and R lines (compare figures 8 and 1). These differences in gene location show that the cytoplasms of 296 A and 2077 A are not identical.

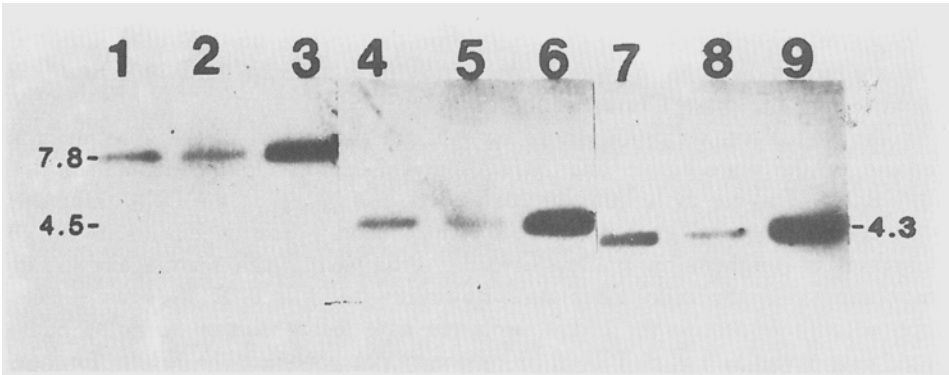

Figure 8. Southern hybridization of the *cox*I gene probe to mtDNA of the male sterile line 296A (lanes 1,4,7), maintainer line 296B (lanes 2,5,8) and the restored line 296R (lanes 3,6,9). mtDNA was digested with *Bam*HI (lanes 1-3), *Hind*III (lanes 4-6) and *Eco*RI (lanes 7-9).

Interestingly, the Southern patterns of *atp*A in 296 A, B and R are identical to 2077 A, B and R. Similarly, the northerns show that the only transcript seen in 296 B of *atp*A is larger than in A and R which possess atleast two transcripts. Thus Southern and northerns for *atp*A are identical in both 2077 and 296 lines (data not shown).

4. Discussion

The male sterile (A) and maintainer (B) have different cytoplasms but identical nucleus and hence analysis of the mitochondrial DNA could bring out differences between the two lines. The restored line (R) carries the cytoplasm of the male sterile line and hence should be identical to it. However, its nuclear genome arises from a combination of the 'A' nucleus and the nucleus from a restorer line. Therefore, if the interaction between the nuclear and mitochondrial genomes results in an organizational change in the mitochondrial genome during multiplication it should be reflected in Southern analysis. Alternatively if there is a nuclear control on the expression of mitochondrial gene (during transcription or post transcription) this should be reflected in the transcript abundance or size. A number of examples of the nuclear control on the transcription and translation of some of the mitochondrial genes are well known. The RF_1 gene, which is one of restorer alleles of the Texas male sterile cytoplasm of maize is known to alter transcription rates of the T-urf gene and lower the abundance of T-urf by as much as 80% (Dewey *et al* 1986) Similarly a maize nuclear gene was recently identified which influences the size and number of transcripts of the mitochondrial *cox*II gene (Cooper *et al* 1990). In

yeasts, the nuclear genes regulating the expression of mitochondrial genes have been identified and cloned. These genes are believed to affect expression through RNA binding proteins encoded by them (Constanzo and Fox 1990; Ackerman *et al* 1991).

The present investigations were undertaken to examine the extent of mitochondrial genome diversity between the male sterile and male fertile lines and to determine whether restoration of the fertility changes the gene location or transcription. Also, as important was the molecular characterization of the sorghum lines used in hybrid seed production in India. Valuable information in relation to these objectives has resulted from the present investigation.

In the first place, the studies have brought out that a part of the mitochondrial genome of the male sterile line 2077 A/296 A is similar to the maintainer line 2077 B/296 B as far as location of *cox*I, *cox*II, *cob* genes on the DNA fragments is concerned. The *cox*I gene has been shown to undergo rearrangements in the 9E cytoplasm of sorghum by Bailley-Serres *et al* (1986a). Such rearrangements are not observed in the milo cytoplasm. However, the A and B lines are indeed different with respect to the genes associated with the production of polypeptides of ATPase complex encoded by the mitochondrial genome. The results for *atp*6 and *atp*9 indicate that the male sterile lines probably possess an additional copy (copies) of these genes. Recently, Mullen *et al* (1992) showed that the male sterile sorghum line of IS1112C has two copies of the *atp6* gene designated as *atp*6-l and *atp*6-2. Both these genes code for a conserved core polypeptide but with diverging amino extensions. The *atp*6-2 version is seen in all sorghum lines examined till date while *atp*6-1 is seen as an additional copy in the A_1, A_2 and A_3 group of male sterile cytoplasms. Similarly, polymorphism has also been observed in plants such as *Brassica,* rice and radish with respect to *atp*6. In most cases, rearrangements in the vicinity of the *atp*6 reading frame resulted in generation of chimeric open reading frames which have been correlated to male sterility (Makaroff *et al* 1989; Kadowaki *et al* 1990; Singh and Brown 1991). In sorghum, however, the northerns do not show any differences between the A and B lines and hence it is unlikely that the additional copy present produces a different kind of a transcript. The presence of similar sizes and numbers of transcripts do not, however, reveal if the transcripts are identical in their translatable regions.

Most interesting is the observation that *atp*A not only shows differences in the Southern but also in northerns between the A and B lines of both 2077 and 296. The Southern data suggest that only one copy of the gene may be present in B lines, as *Bam*HI and *Sma*I produce only one band, whereas *Hind*III produces two bands of unequal intensities. However, the presence of two complete copies of the gene in Kafir and Milo cytoplasms has been shown by careful analysis of the *atp*A reading frames by earlier workers (Bailley-Serres *et al* 1986b). Hence, it is possible that, in 2077B, there might be two copies in tandem without any site for *Bam*HI and *Sma*I inbetween. The A and R lines of 2077 and 296 probably possess an additional copy of *atp*A and one of these has a site for *Bam*HI and *Hind*III within it. The northerns show a different sized transcript for the B lines of 2077 and 296. The transcript is larger by about 300 nucleotides in both the cases. This suggests that either the translatable region is larger, producing a bigger polypeptide, or that the 5' and/or 3' untranslatable region of B is different than in the A and R lines. Such differences could result in altered gene expression. This was recently

shown to be the case for the sugarbeet *atp*A genes of the male sterile (TK81-MS) and its maintainer line (TK81-0) by Senda *et al* (1993) where the two genes diverge completely 47 bp upstream of the initiation codon. On the other hand the TK81-0 and another CMS line were shown to diverge 399 bp downstream of the termination codon. Both rearrangements led to differences in the transcript pattern. Such rearrangements and transcript differences have also been shown in radish and sunflower (Makaroff *et al* 1990; Kohler *et al* 1991).

The ATPase complex consists of two major components; the F_o which is membrane bound and F_1 which is attached to the F_o and faces the matrix. Each consists of several polypeptides. Of the different components of F_o, polypeptides encoded by *atp*6 and *atp*9 are contributed by the mitochondria. Since the transcripts relating to these two are identical in A, B and R lines, we believe that the products of the *atp*6 and *atp*9 may not be functionally and structurally different between A and B and hence should not lead to an altered but inefficient assembly of F_o that could cause male sterility. It is important to note that although there are some differences between 2077 and 296 in the Southerns of *atp*6 and *atp*9*,* the northerns are identical.

We had earlier proposed that multisubunit complexes of the mitochondrial membranes may not assemble properly if the subunits encoded by the mitochondrial genome are incompatible with the complementary subunits produced by the nuclear genome (Sane and Nath 1993). Compatibility depends upon providing an appropriate (complementary) subunit to make the ATPase enzyme. The defective assembly, even if functional, would be inefficient and hence could lead to male sterility. The F_1 component of the ATPase complex consists of 5 subunits, α, β, γ, δ and ε. Of these α is the only one encoded by the mitochondrion *(atp*A) and it interacts with the β subunit (which is almost as large as the α subunit) imported from the nucleus. In the B line, however, the larger transcript might be yielding an α subunit which forms a perfect assembly with the complementary subunits of F_1 imported from the nucleus. If so, this should be reflected in the functional characteristics of F1. Preliminary results do suggest that the enzyme activity of F_1 ATPase, and its kinetic properties, are different between the sterile 2077A and the fertile 2077B lines (manuscript in preparation).

If the above proposal is true, one has to explain how the identical α subunit in the restored line forms an efficient assembly of F_1 resulting in restoration of fertility. This can happen if the different nuclear background in the R line contributes the four complementary subunits (β, γ, δ and ε) that are compatible with α subunit of the R line (identical cytoplasm as of A line). Considering the fact that α subunit primarily interacts with a large β subunit (Thomas *et al* 1992) it can be proposed that 'restoration' is in fact related to providing a compatible β subunit through change in the nuclear genome or background. This should result in the restoration of the function of F_1 in the restored line although the α subunit is identical to the one in A line. The restoration of fertility in both the cases (hybrid seeds of 2077 and 296) also restores ATPase function (data not presented) which is similar to the respective fertile lines. Our preliminary results show that the kinetics of F_1 ATPase in the restored (fertile) line are similar to the maintainer fertile B line although its α subunit is identical to A line.

Whereas restoration in these lines may relate to restoration of the ATPase complex, it may not be restricted to this gene alone. It is likely that there may

be other functions relating to other genes that may be restored but have not been detected by us. It is believed that there may be up to three restorer genes involved in fertility restoration in sorghums (A R Dabholkar, personal communication). We propose that one of these relates to restoration of ATPase function and specifically relating to *atp*A gene. To confirm this proposal we have undertaken studies on several lines of CMS produced by Indian breeders and the results (manuscript in preparation) support the above proposal.

Acknowledgements

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