MITOCHONDRIA DNA RESTRICTION ENDONUCLEASE PATTERNS IN SORGHUM

by

HSING CHUNG LEE

B.A., Tung-Hai University, 1982

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

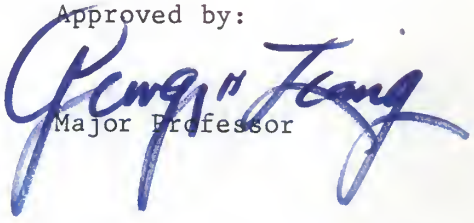
MASTER OF SCIENCE

Department of Genetics

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1986

Approved by:



Major Professor

ACKNOWLEDGMENTS

I would like to express my sincere appreciation to my major advisor Dr. George H. Liang for his support and guidance. I thank Dr. Subbaratnam Muthukrishanan for his assistance and kindly letting me use his laboratory. I would also like to thank Dr. Paula Bramel-Cox for the seeds used in this research and Dr. Gary Paulsen for reviewing the manuscript. Appreciation also goes to members in laboratories and friends in Kansas State University for their stimulating discussions and friendships.

I am deeply grateful to my mother, Chia-Yin Lee, my family for being a constant source of affection, encouragement and joy; and to my wife, Grace, for her support and inspiration.

TABLE OF CONTENTS

	Page
Abstract	1
Introduction	2
Material and method	6
Results	12
Discussion	17
References	20
Figures	23

LIST OF FIGURES

FIGURE PAGE

1. Agarose gel electrophoretic patterns of Ecor I digestion of mt-DNA from KS34 A through KS39 A 23

2. Agarose gel electrophoretic patterns of Ecor I digestion of mt-DNA from Sumac, KS5 A & B, Redlan A & B,Martin A & B, KS45 A & B, KS34 A 24

3. Agarose gel electrophoretic patterns of Hind III digestion of mt-DNA from Sumac, KS5 A & B, Redlan A & B,Martin A & B, KS45 A & B, KS34 A 25

4. Agarose gel electrophoretic patterns of BamH I digestion of mt-DNA from Sumac, KS5 A & B, Redlan A & B,Martin A & B, KS45 A & B, KS34 A 26

5. Agarose gel electrophoretic patterns of EcoR I digestion of mt-DNA from S. halepense Redlan A & B, S. versicolor 27

6 Agarose gel electrophoretic patterns of EcoR I digestion of mt-DNA from S. halepense KS5 A & B, S. versicolor 28

ABSTRACT

Mitochondrial DNAs (mt-DNA) were isolated from Sorghum versicolor (2n=10), S. halepense (2n=40) and 11 male sterile, 5 male fertile lines of S. bicolor. Restriction endonucleases EcoR I, Hind III, and BamH I were used. Restriction patterns were compared by agarose gel electrophoresis.

EcoR I digestion showed that S. versicolor had a unique mt-DNA restriction pattern that was completely different from other sorghum mt-DNA restriction patterns examined. There were some similarities among sorghums that had chromosome numbers 2n=20 and 2n=40. Sumac and KS5 B had distinct fragments in all three digestions, showing that their cytoplasms were different from other lines to a certain degree. S. halepense had almost the exact restriction pattern as S. bicolor. These observations agreed with cytogenetic and morphology observations suggesting that the cytoplasm of S. versicolor was distinctly different from other sorghums examined and the cytoplasm of S. halepense is extremely similar to S. bicolor.

The mt-DNA restriction patterns of cytoplasmic male sterile lines differed, consistently and in the same region, from those of male fertile lines in specific bands depending on the enzymes used. The differences between male sterile and fertile lines are probably attributable to the specific region (or regions) that is related to cytoplasmic male sterility.

INTRODUCTION

The grass genus Sorghum is one of immense morphological variation. It is usually divided into 5 subsections: Chaetosorghum, Heterosorghum, Parasorghum, Stiposorghum and Sorghum. Only Sorghum seems to have contributed towards the origin of cultivated sorghum. This subsection was divided into 3 species: S. bicolor, S. propinquum, and S. halepense. S. bicolor was further divided into 3 subspecies: bicolor, arundinaceum and drummondii. Cultivated sorghum, S. bicolor subspecies bicolor is divided into 5 races: bicolor, kafir, caudatum, durra, guinea. The subspecies S. bicolor subspecies arundinaceum is divided into 4 races: arundinaceum, virgatum, aethiopicum, verticilliflorum (de Wet, 1978). The classification is complicated by the diversity and variability of the Sorghum genus.

Cytogenetic analysis has been used to evaluate the relationship among species of Sorghum and it showed that S. halepense chromosome (2n=40) morphology was very similar to the morphology of S. bicolor (2n=20) but S. versicolor had distinct chromosome morphology (Gu et al., 1984). Analyses of cytoplasmic relationships among S. halepense, S. versicolor, and S. bicolor could provide another aspect to understand their relationships.

Mitochondrial DNA (200-2400 kb) in higher plants (Levings, 1983), much larger and more variable than chloroplast DNA, is a good subject to analyze the cytoplasmic relationship among Sorghum. Mitochondrial DNA (mt-DNA) restriction pattern analysis showed that it is possible to distinguish milo mt-DNA from kafir

mt-DNA; both belong to S. bicolor (Pring et al., 1982(a)).

The study of cytoplasmic relationship could help plant breeders to increase cytoplasmic diversity that would reduce the probability of epidemics arising from cytoplasmically inherited susceptibility to disease. The basis of sorghum improvement in the USA has been the milo x kafir cross and natural crosses selected by farmers. Martin, Redlan and KS45, subjects of this study, belong to this category and the mt-DNA restriction patterns have not been studied. KS34 A through KS39 A, developed to broaden the cytoplasmic basis, are male sterile lines essentially differing only in cytoplasms (Ross and Hackerott, 1972). Conde et al. (1982) compared the mt-DNA restriction patterns of KS 34A through KS 39A and found the variation was limited.

Sumac, imported from Africa, that could have a cytoplasm different from that of cultivated sorghums, is another interesting subject to study. KS 5A and KS 5B, both were selections from Sumac, are also included in this study.

Considerable molecular level comparisons have been made between cytoplasmic male sterile and male fertile cytoplasms of sorghum in order to understand cytoplasmic male sterility (CMS). CMS, a maternally inherited factor that causes failure to produce functional pollen, is found in a wide range of plant species and is used commercially in the production of hybrid seed, by preventing self fertilization of the seed parent in such crops as maize (Zea mays), sorghum (Sorghum bicolor), sugar beet (Beta vulgaris), sunflower (Helianthus annuus), etc. (Leaver and Gray, 1982).

It is believed that CMS is encoded in mitochondria (Hanson and Conde, 1985). Studies of chloroplast and mitochondrial DNA restriction patterns showed that the mitochondrial DNA restriction patterns from CMS lines were distinguishable from those of fertile lines, while the chloroplast DNA patterns were indistinguishable (Hanson and Conde, 1985). Differences in the proteins synthesized by mitochondria from different CMS types have been demonstrated for both maize and sorghum (Leaver and Gray, 1982). These findings strongly suggested that genetic determinants controlling CMS in these species are located in mitochondria.

Restriction endonuclease analysis of sorghum mt-DNA showed that in six cases mt-DNA restriction patterns were correlated with observed differences in fertility and cytoplasmic background (Conde et al., 1982). One sorghum CMS cytoplasm (IS112C) was found to have two linear plasmid-like DNAs, N1 (5.7 kb), N2 (5.3 kb) (Pring et al., 1982(b)); both were similar to S1 and S2 plasmid-like DNAs found in CMS maize (Kemble et al., 1980). Many efforts have been made to understand the relationship between CMS expression and the plasmid-like DNAs found in mitochondria. To date there exists no genetic or molecular evidence in sorghum which would implicate these molecules in control of CMS expression in a manner analogous to S1 and S2 in maize (Chase and Pring, 1986).

A few reports, none in sorghum, concern comparisons of mt-DNA restriction patterns between CMS and fertile lines. The lack of isogenic CMS/male fertile lines limited this kind of comparison, an other reason is the difficulty of isolating mt-DNA

suitable for restriction pattern analysis. The mt-DNA restriction patterns of a pair of sugar beet lines that were nearly isogenic except for the CMS/fertile character have been compared (Powling, 1982). There were two types of mt-DNA, one associated with fertile and the other with CMS sugar beet.

Sorghum CMS lines (A lines) are selections from normal fertile lines (B lines) and the only genetic difference between CMS and fertile lines is the male fertility. If relationships exist between CMS and mt-DNA, they could be revealed by comparison of mt-DNA restriction patterns of CMS lines and male fertile lines. When mt-DNA restriction patterns of A lines differ consistently from those of B lines, it is probable that the differences of the specific mt-DNA regions are related to the differences in male fertility. Comparing the restriction patterns among fertile and CMS sorghums could provide further understanding of CMS in molecular level.

The objectives of this study are:

1. To develop a rapid method that requires limited amount of seeds to isolate sorghum mt-DNA which is suitable for restriction pattern analysis.
2. To compare mt-DNA restriction patterns among different sorghum species, including those of different ploidy levels, in order to understand their cytoplasmic relationships.
3. To compare mt-DNA restriction patterns of isogenic male-fertile and cytoplasmic male sterile sorghums for a better understanding of the molecular basis of CMS.

MATERIALS AND METHODS

Plant materials:

S. halepense (2n=40), *S. bicolor* (2n=20) including nine CMS lines (A lines): KS5 A, KS34 A, KS35 A, KS36 A, KS37 A, KS38 A, KS39 A, KS45 A, Redlan A, Martin A and five male fertile lines (B lines): Sumac, KS45 B, KS5 B, Redlan B, Martin B in section *Sorghum*, and *S. versicolor* (2n=10) in section *Parasorghum* were used in this study. KS5 A and KS5 B were selections from Sumac. Since each CMS line was selected from an individual normal fertile line, both lines would be isogenic, the only difference is their male fertility.

Due to the difficulty of germinating *S. versicolor* and *S. halepense* and limited availability of seeds, *in vitro* germination on a culture medium was made. About 100 seeds were freed of glumes and washed with 50% sodium hypochloride for 10 minutes, rinsed with tap water for one hour and soaked in 70% ETOH for 2 minutes, then rinsed again with 10X volume of autoclaved water. The seeds were then placed on the surface of a culture medium. The 500 ml culture medium contains Murashige and Skoog (1962) inorganic salts, 10 g sucrose, 0.05 mg thiamine-HCl, 0.5 mg glycine, 0.25 mg nicotinic acid, 0.25 mg pyridoxine-HCl, 50 mg myo-inositol, 1.25 mg 2.4-dichlorophenoxyacetic acid, 0.025 mg kinetin, 1.1 mg zeatin and 2.9 g Difco agar (Smith et al., 1985). The seeds were incubated in a growth chamber for 1 to 4 weeks in darkness at 27^{o}C.

For other sorghum species, 6 to 10 grams of seeds were used for each mt-DNA extraction. Seeds were sterilized described above and placed on an autoclaved Petri dish with two layers of moistened paper towel and kept in darkness for 5 to 6 days at 24°C The etiolated mesocotyl and coleoptile tissue were harvested and weighed before homogenization.

Isolation of mitochondria:

Mitochondrial DNA was isolated at 4°C by a modified procedure of Kemble et al. (1980). Approximately 5 to 10 grams of etiolated seedings were homogenized for 2 minutes using a motar and pestle. For every gram of fresh tissue, 3 volumes of isolation buffer [0.5 M Manitol, 0.01 M N-Tris Hydroxymethyl 1-2-aminoethane sulfonic acid (pH 7.2), 0.005 M EDTA (pH 8.0), 0.15% BSA and 0.05% cysteine] were used. The homogenate was filtered through 4 layers of cheesecloth and 1 layer of Miracloth, both having been presoaked with isolation buffer, into a 50-ml centrifuge tube. The following centrifugations were carried out in a Sorvall centrifuge using an SS-34 rotor at 4°C. The filtrate was centrifuged at 1,000 X g (3,000 RPM) for 10 minutes and the resulting supernatant was centrifuged at 12,000 X g (11,000 RPM) for 20 minutes to obtain crude mitochondrial pellet. The pellet was gently resuspended in 10 ml isolation buffer using a small paint brush and centrifuged at 1,000 X g for 10 minutes. The supernatant was then treated with DNase.

DNase treatment:

Magnesium chloride and DNase were added to final concentrations of 0.01 M and 10 µg/g fresh tissue, respectively. The reaction was carried out for 1 hour on ice and terminated immediately by subjecting the solution to sucrose gradient purification.

Sucrose gradient purification:

The DNase-treated solution was layered on top of 20 ml washing buffer [0.6 M sucrose, 0.01 M TES (pH 7.2), 0.02 M EDTA (pH 8.0)] and centrifuged at 14,000 X g for 20 minutes. The pellet was resuspended in 10 ml washing buffer and centrifuged at 750 X g (2,500 RPM). The supernatant was then centrifuged twice at 12,000 X g for 20 minutes. The pellet contained the mitochondria.

Lysis of Mitochondria:

The mitochondrial pellet was resuspended in 2 ml of lysis buffer [0.05 M Tris-HCl (pH 8.0), 0.01 M EDTA (pH 8.0), 2% Sarkosyl, 0.02% autodigested (for 1 hour) Proteinase K] and incubated at 37^{o}C for 1 hour with gentle shaking (40 RPM).

Purification of Mitochondrial DNA:

The lysate was made 0.5 M with respect to ammonium acetate in a 15-ml Kimax tube and an equal volume of phenol was added and mixed gently for two minutes. Equal volume of chloroform (chloroform : isoamyl alcohol=24 : 1) was then added to the solution and mixed for 2 minutes. The Kimax tube was centrifuged at 1,900 X g (4,000 RPM) for 10 minutes at 24°C. The upper aqueous phase was transferred to a fresh tube and the phenol/chloroform extraction was repeated 3 times.

Ethanol precipitation of mitochondrial DNA:

Two volumes of 100 % ethanol were added to the final aqueous phase in a siliconized (see appendix) 15-ml Corex tube and the tube was mixed and stored overnight at -20°C. The DNA pellet was harvested by centrifuging at 12,000 X g at 4°C for 25 minutes. The pellet was washed by adding 1.4 ml 70% ethanol to the Corex tube and incubated 10 minutes at 37°C. The mixture was transferred to a 1.5-ml Eppendorf microcentrifuge tube and centrifuged at 14,000 X g, 4°C for 25 minutes. The supernatant was removed and the DNA pellet was vacuum dried for 7 minutes and the purified mt-DNA was resuspended in 30-80 µl of 0.01 M Tris-HCl (pH 8.0) and 0.0005 M EDTA pH 8.0. For enzymes other than EcoR I to digest the mt-DNA properly, the DNA resulting from ethanol precipitation needed to be further purified by spermine precipitation.

Spermine precipitation of mitochondrial DNA:

Spermine was added to the DNA sample to the final concentration of 100 mM. The solution was mixed gently and kept at 4°C for 15 minutes. The solution was then centrifuged at 14,000 X g, 4°C for 20 minutes and the supernatant was carefully removed. Spermine extraction buffer [75% ethanol, 0.3 M sodium acetate, 0.01 M magnesium acetate] was added to the DNA pellet. The solution was mixed gently every 15 minutes and kept at 4°C for one hour. The solution was centrifuged at 14,000 X g, 4°C for 20 minutes and the supernatant was removed. The DNA pellet was vacuum dried for 7 minutes and resuspended in the original volume of 0.01 M Tris-HCl (ph 8.0) and 0.0005 M EDTA (pH 8.0).

Restriction endonuclease digestions:

The restriction enzymes were purchased from Bethesda Research Laboratories, Inc.. All restriction enzymes except EcoR I were used according to the supplier's recommendations.

The digestion condition for EcoR I was 1 mM DTT, 2 mM Spermidine, 100 mM NaCl, 50 mM Tris-HCl (pH 8.0), 0.25 µg/ul BSA. The digestion condition for Hind III was 50 mM was 50 mM NaCl, 10 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 0.25 ug/ul BSA. The digestion condition for BamH I was 20 mM Tris-HCl (pH 8.0), 100 mM NaCl 10 mM $MgCl_2$, 0.25 µg/ul BSA.

5 to 6 ug of mt-DNA were digested in 20 ul reaction with 20 to 40 units of restriction enzyme and incubated at 37°C for 6 hours. The reaction was terminated by adding 5 µl loading dye:

0.25% bromophenol blue, 0.25% xylene cyanol 25% Ficoll (type 400) (Maniatis et al., 1982), and stored immediately at -20°C.

Agarose gel electrophoresis:

Digested mt-DNA fragments were separated by electrophoresis in 0.7% agarose horizontal slab gel at 3 v/cm for 17 hours at room temperature in TBE buffer [0.089 M Trisma base, 0.089 M Boric acid, 0.002 M EDTA]. The gel was stained with 0.6 µg/ml Ethidium bromide for 20 minutes and destained for 20 minutes in deionized water. The gel was placed on top of a UV transilluminator and the restriction pattern was photographed through Kodak Wratten No. 9 and No. 23 filters with Type 55 P/N Polaroid film.

RESULTS

Yields of mitochondrial DNA:

An average of 25 µg of mt-DNA was obtained but the amount varied with the sorghum species. Mt-DNA restriction patterns obtained by this method were as good as those obtained by others using more complicated methods.

Native mt-DNA analysis:

Seventeen sorghum mt-DNA entries, 10 CMS lines and 7 male fertile lines, were analyzed by electrophoresis in 1% agarose gel without restriction endonuclease digestion. These entries included all the sorghum listed in the materials. None had plasmid-like DNAs. Previous studies also showed that very few sorghums have plasmid-like DNAs in their mitochondria (Pring et al., 1982(b)).

Effects of spermine precipitation on restriction enzyme activity:

Mt-DNA isolated by ethanol precipitation was suitable for EcoR I digestion, but other restriction enzymes failed to digest it properly, as indicated by smeared electrophoretic patterns on agarose gels. The mt-DNA to be digested by restriction enzymes other than EcoR I was then subjected to spermine precipitation for further purification. This procedure probably removed some

of the salts or other impurities that were trapped in the mt-DNA pellet that would make the restriction enzymes Hind III and BamH I not function properly.

Mt-DNA restriction patterns similarity among KS34 A through KS39 A:

The EcoR I restriction patterns of KS34 A through KS39 A (Fig. 1) are almost identical. Only KS39 A (lane 6) had one unique band, just below 9.4 kb, which is very faint compared to other bands.

Comparison of mt-DNA among S. bicolor:

A. EcoR I digestions:

The EcoR I digestion patterns (Fig. 2) of Redlan B, Martin B and KS45 B (lanes 3, 4, and 5) were identical. Sumac and KS5 B (lanes 1 and 2) were distinguished from the other male fertile lines by having 3 new fragments (small and medium arrows) and 1 band was missing (small arrows). Redlan B (lane 3) had two very faint bands.

The EcoR I digestion patterns of CMS lines: KS5 A, Redlan A, Martin A, KS45 A, KS34 A (lanes 6, 7, 8, 9, and 10) were extremely similar except that KS5 A (lane 6) had one extra band just above 9.4 kb.

All the CMS lines (lanes 6, 7, 8, 9, and 10) could be distinguished from male fertile lines (lanes 1, 2, 3, 4, and 5) in the

same region about 6.4 kb (large arrows).

B. Hind III digestions:

The Hind III digestions patterns (Fig. 3) of CMS lines: KS5 A, Redlan A, Martin A, KS45 A, and KS34 A (lanes 6, 7, 8, 9, and 10) were almost identical. All the CMS lines had three extra bands (large arrow) that none of the male fertile lines had (lanes 1, 2, 3, 4, and 5).

Sumac and KS5 B (Lanes 1 and 2) differed from other fertile lines (lanes 3, 4, and 5) in many bands (medium arrows). Martin B and KS45 B had one extra band (lanes 4 and 5, small arrow).

C. BamH I digestions:

The BamH I digestions (Fig. 4) produced many large fragments that were difficult to separate even in a 0.7% agarose gel. However, it was possible to recognize some of the important differences. The BamH I restriction patterns were very similar among CMS lines except that KS45 A (lane 9, small arrow) had one extra band, and one band was missing in KS5 A (lane 6, small arrows). In the male fertile lines, Sumac and KS5 B continued to be different from other fertile lines by missing one band (lanes 1 and 2, small arrow).

In BamH I digestions, the differences between CMS and male-fertile lines continued to follow the patterns of EcoR I and Hind III. The CMS lines (lanes 6, 7, 8, 9, and 10) had two extra bands and one band was missing.

Comparison of restriction patterns from different germinating conditions:

The limited source of seeds and difficulty of germinating S. halepense and S. versicolor prompted the idea of germinating the seeds on a nutrient medium. Seedlings grown on the medium in darkness increased the amount of tissue per seed, avoid chloroplast DNA contamination, and retained the softness of the tissue which is essential for the isolation of sorghum mt-DNA with this particular method.

There might be concerns that culture medium may alter mt-DNA. Sumac was used as the standard sample to study the effect of culture medium on mt-DNA. Sumac seeds were germinated on a culture medium and also in Petri dishes. The mt-DNAs, isolated from Sumac that had been germinated in the two different conditions, were digested by EcoR I and analyzed by agarose gel electrophoresis. The two restriction patterns were exactly the same indicating that alteration of sorghum mt-DNA was not induced by the culture medium. This observation supports the previous observation of Nagy et al. (1983) that mt-DNA alteration is not induced by culture medium.

Comparison of mt-DNA among Sorghums of different ploidy level :

Mt-DNAs were isolated from etiolated seedings of S. versicolor (2n=10) and S. halepense (2n=40), germinated in culture medium and digested by EcoR I. The EcoR I restriction pattern of

S. halepense (Fig. 5, lane 1) was similar to Redlan B and Redlan A (Fig. 5, lanes 2 and 3) that were the representatives of sorghums having chromosome number 2n=20 but there were some unique regions that only S. halepense had (Fig. 5, small arrows).

It is important to note that the EcoR I restriction pattern of S. halepense had the bands that characterized the male fertile lines (Fig. 5, large arrows). The EcoR I restriction pattern of S. versicolor was completely different from other sorghum examined (Fig. 5, lane 4).

The EcoR I restriction pattern of S. halepense similar to KS5 B (Fig. 6, lane 1) but it had extra bands (Fig. 6 upper small arrow) and did not have the band near 4.3 Kb (Fig. 6, lower small arrow).

S. versicolor, on the other hand, had its own unique mt-DNA restriction pattern that did not resemble the mt-DNA restriction pattern of any other sorghums in this study (Fig. 6, lane 4).

DISCUSSION

The procedure of isolating sorghum mt-DNA used in this study requires less time, equipments, and chemicals than other known procedures. The restriction patterns were clear enough to resolve most of the large fragments, but the small fragments, less than 1 kb, were not separated clearly in a 0.7% agarose gel.

There have been many efforts to relate plasmid-like DNAs to CMS (Chase and Pring, 1986). However, plasmid-like mt-DNAs were not observed in this study. At present, no conclusion can be drawn regarding the role of plasmid-like mt-DNAs in cytoplasmic male sterility of sorghum.

The mt-DNA restriction patterns of 20-chromosome sorghums were very similar except Sumac, KS5 A, and KS5 B. KS45 B and Martin B had one extra band than others in Hind III digestions and KS45 A had one extra band in BamH I digestion. Sumac, KS5 A and KS5 B were clearly distinguishable from others based on the restriction patterns obtained from each of the three restriction enzymes. Therefore, use of Sumac, KS5 A or KS5 B cytoplasms could increase the cytoplasm diversity of sorghum and help avoid vulnerability to cytoplasmic-related disease outbreaks.

The mt-DNA restriction patterns of S. halepense (2n=40) showed a very close resemblance to the patterns of 20-chromosome sorghums. The mt-DNA restriction pattern of S. versicolor was distinct from all other sorghums. This observation was consistent with the karyotype analysis (Gu et al., 1984) and the morphological studies (Doggett, 1976).

The very faint bands resulting from EcoR I digestion of Redlan B, Hind III digestions of KS45 B and Martin B, and BamH I digestion of KS45 A, could be the result of minor mt-DNA populations or nucleotide modifications that occur in small stoichiometries.

The results from all three restriction enzyme digestions showed that there were consistent differences between CMS and male fertile lines. There were specific bands in all three digestions that characterized male fertile lines and CMS lines. Although Sumac and KS5 B differed from other 20-chromosome male fertile lines in many bands but they had the bands that characterized male fertile lines. KS5 A, on the other hand, resembled CMS lines. These observations suggest that a specific alteration or alterations in mt-DNA is related to CMS in sorghum. When comparing specific regions, regions that distinguish CMS from male fertile lines, in the mt-DNA restriction patterns of different sorghum lines it is always possible to divide them into two groups: CMS and male fertile. The mt-DNA alterations involved large mt-DNA region or regions since all three enzymes detected the difference. The alterations in restriction patterns can not be explained by a single mutation. A concerted mt-DNA rearrangement could produce the alterations observed in sorghum mt-DNA.

It might have been suspected that the mt-DNA of CMS lines tested could have originated from one cytoplasm. The observed differences in mt-DNA could then be the result of their different origins and not, in fact, have a causal relationship with the CMS trait. However, as the materials and methods pointed out

this is not likely the case. Further studies, such as blotting and *in vitro* translation, will be needed to prove that mt-DNA is indeed responsible for CMS.

REFERENCES

1. Chase, C. D. and D. R. Pring. 1986. Properties of the linear N1 and N2 plasmid-like DNAs from mitochondria of cytoplasmic male-sterile Sorghum Bicolor. Plant Molecular Biology 6: 53-64.

2. Conde, M. F., D. R. Pring, K. F. Schertz, and W. M. Ross. 1982. Correlation of mitochondrial DNA restriction endonuclease patterns with sterility expression in six male-sterile sorghum cytoplasms. Crop Sci. 22: 536-539.

3. de Wet, J. M. J. 1978. Systematics and evolution of Sorghum sec. Sorghum. American J. of Botany 65 (4): 477-484.

4. Doggett, H. 1976. (Sorghum) p. 112-117. In N. W. Simonds (ed.) Evolution of crop plants. Longman, London & New York.

5. Gu, M. H., H. T. Ma, and G. H. Liang. 1984. Karyotype analysis of seven species in the genus Sorghum. The Journal of Heredity 75: 196-202.

6. Hanson, M. W. and M. F. Conde. 1985. Functioning and variation of cytoplasmic genomes: lessons from cytoplasmic-nuclear interactions affecting male sterility in plants. International Review of Cytology 94: 213-267.

7. Kemble, R.J., R. E. Gunn, and R. B. Flavell. 1980. Classification of normal and male-sterile cytoplasms in maize. Genetics 95: 451-458.

8. Leaver C. J. and M. W. Gray. 1982. Mitochondria genome organization and expression in higher plant. Annual Review of Plant Physiology 33: 373-402.

9. Levings, C. S. III. 1983. The plant mitochondrial genome and its mutants. Cell 32: 659-661.

10. Maniatis, T., E. F. Fritsch and J. Sambrook. 1982. Molecular cloning p. 455. Cold Spring Harbor Laboratory, New York.

11. Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15: 473-497.

12. Nagy F., G. Lazar, L. Menczel, and P. Maliga. 1983. A heteroplasmic state induced by protoplast fusion is necessary condition for detecting rearrangements in Nicotiana mitochondrial DNA. Theor. Applied Genetics. 66: 203-207.

13. Powling A. 1982. Restriction endonuclease analysis of mitochondrial DNA from sugarbeet with normal and male-sterile cytoplasms. Heredity 49: 117-120.

14. Pring D. R., M. F. Conde, and K. F. Schertz. 1982(a). Organelle genome diversity in sorghum: Male-sterile cytoplasm. Crop Sci. 22: 414-421.

15. Pring D. R., M. F. Conde, K. F. Schertz, and C. S. Leving, III. 1982(b). Plasmid-like DNAs associated with mitochondria of cytoplasmic male-sterile Sorghum. Molecular Gene and Genetics. 186: 180-184.

16. Ross, W. M. and H. L. Hackerott. 1972. Registration of seven isocytoplasmic Sorghum germplasm lines. Crop Science 12: 720.

17. Smith R.H., S. Bhaskaran, and F. R. Miller. 1985. Screening for drought tolerance in sorghum using cell culture. In Vitro Cellular & Development Biology 21: 541-545.

Fig. 1. Agarose gel electrophoretic patterns of EcoR I digestion of mt-DNA from CMS lines: KS34 A (1), KS35 A (2), KS36 A (3), KS37 A (4), KS 38 A (5), KS39 A (6). Molecular weight marker, bacteriophage lamda DNA digested by EcoR I (M).

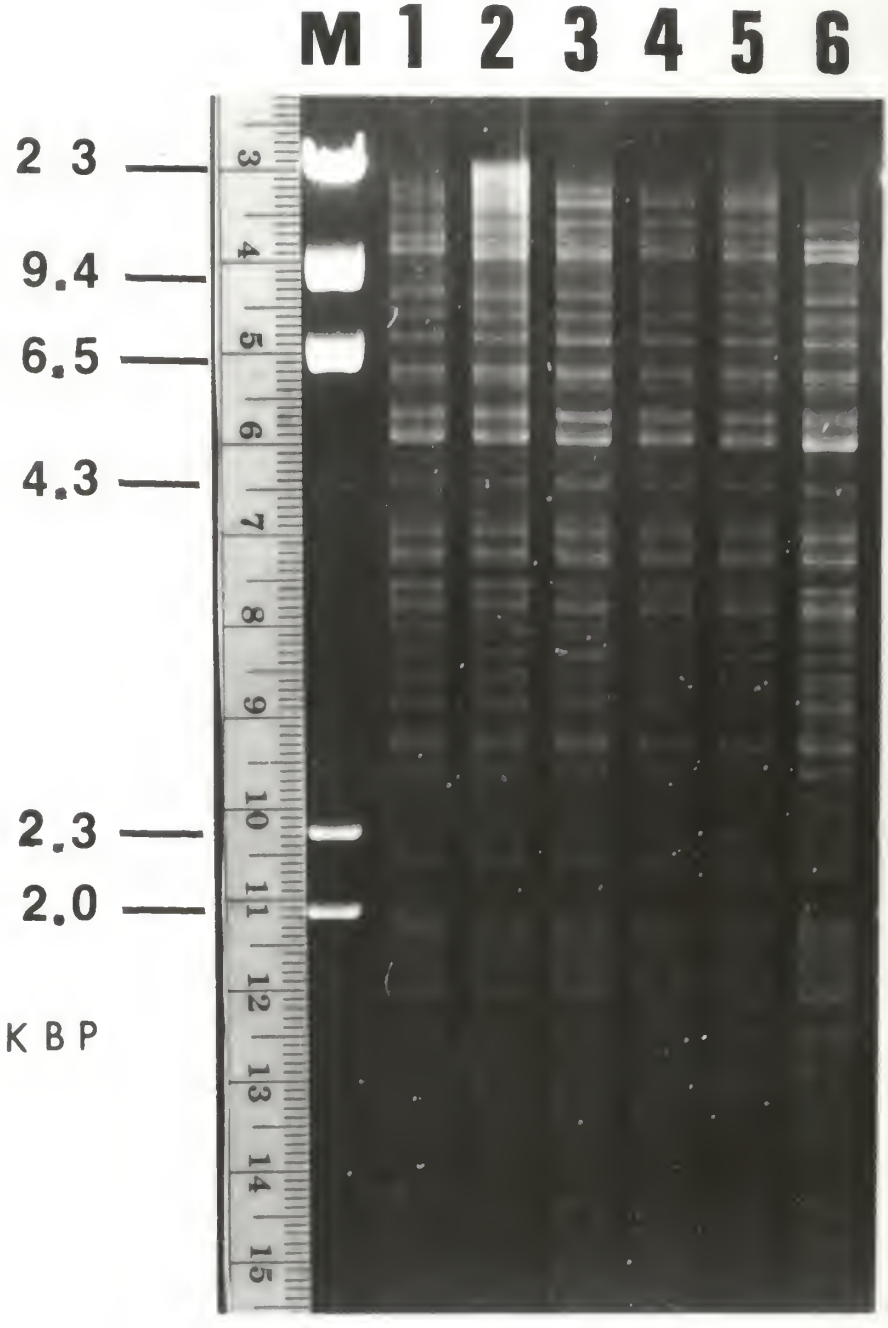

M 1 2 3 4 5 6
2 3
9.4
6.5
4.3
2.3
2.0
K B P

Fig. 2. Agarose gel electrophoretic patterns of EcoR I digestion of mt-DNA from male-fertile lines: Sumac (1), KS5 B (2), Redlan B (3), Martin B (4), KS45 B (5), and CMS lines: KS5 A (6). Redlan A (7), Martin (8), KS45 A (9), KS34 A (10). Molecular weight marker, bacteriophage lamda DNA digested by EcoR I (M).

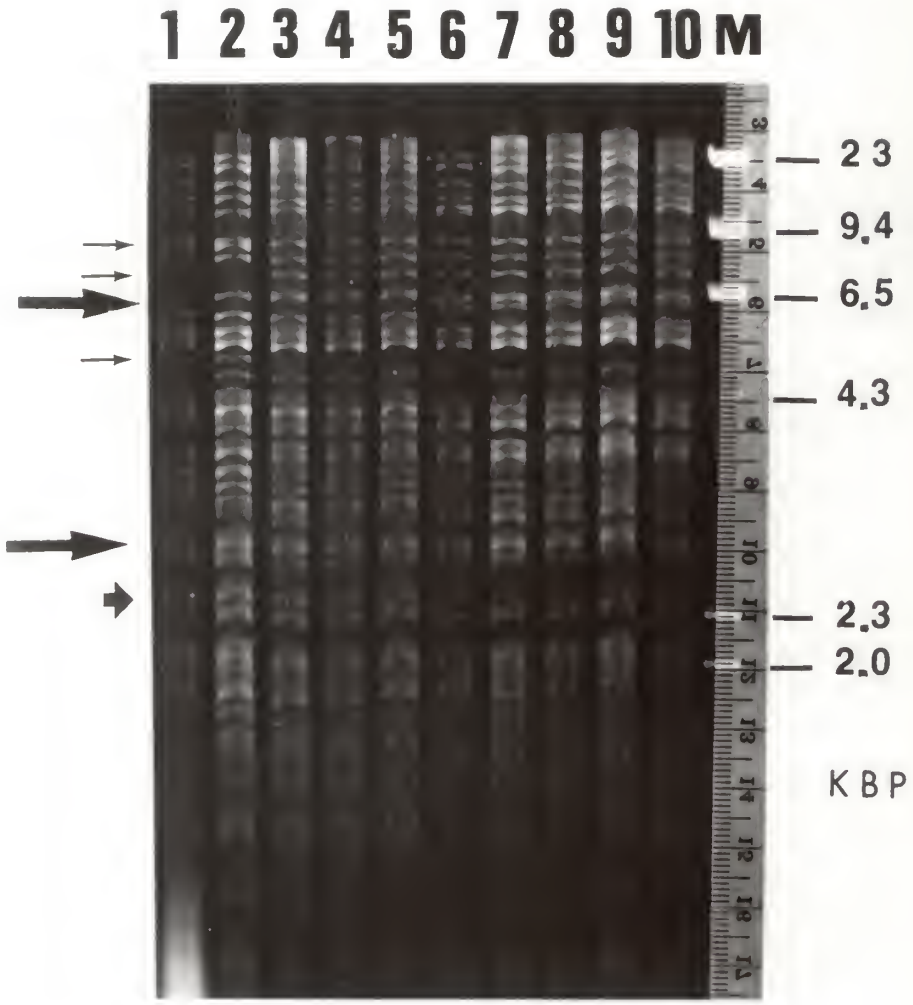

1 2 3 4 5 6 7 8 9 10 M
23
9.4
6.5
4.3
2.3
2.0
KBP

Fig. 3. Agarose gel electrophoretic patterns of Hind III digestion of mt-DNA from male fertile lines: Sumac (1), KS5 B (2), Redlan B (3), Martin B (4), KS45 B (5), and CMS lines: KS5 A (6), Redlan A (7), Martin A (8), KS45 A (9), KS34 A (10). Molecular weight marker, bacteriophage lamda DNA digested by EcoR I (M).

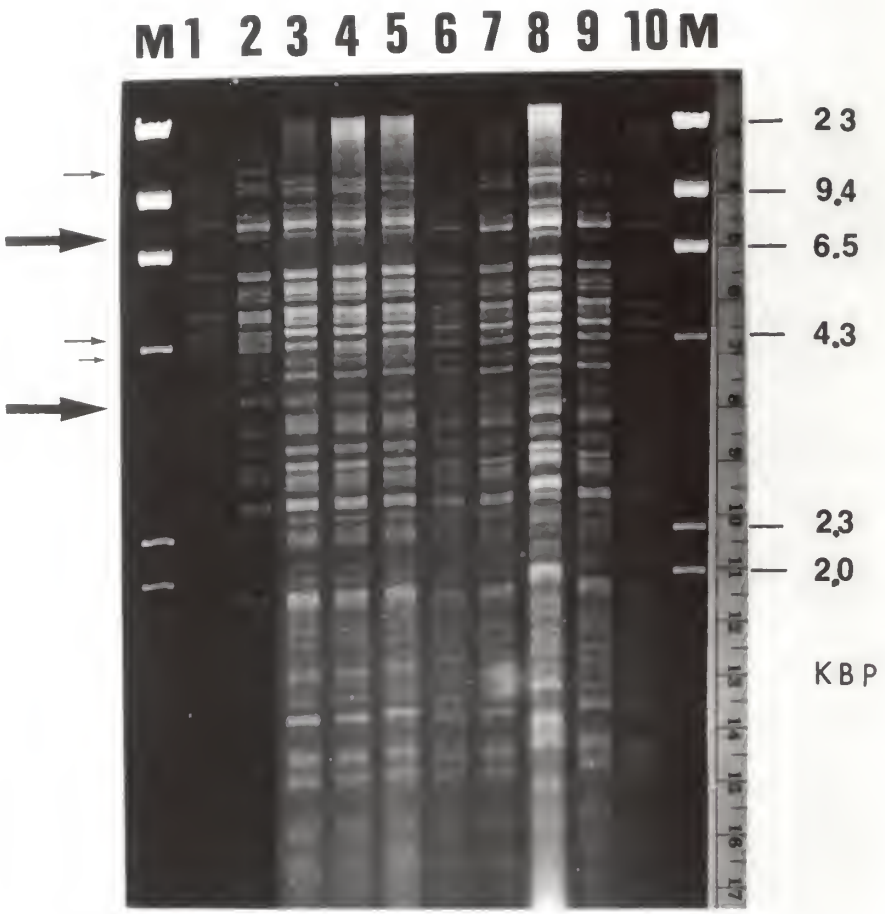

M 1 2 3 4 5 6 7 8 9 10 M
23
9,4
6,5
4,3
2,3
2,0
KBP

Fig. 4. Agarose gel electrophoretic patterns of BamH I digestion of mt-DNA from male fertile lines: Sumac (1), KS5 B (2), Redlan B (3), Martin B (4), KS45 B (5), and CMS lines: KS5 A (6), Redlan A (7), Martin (8), KS45 A (9), KS 34 A (10). Molecular weight marker, bacteriophage lamda DNA digested by EcoR I (M).

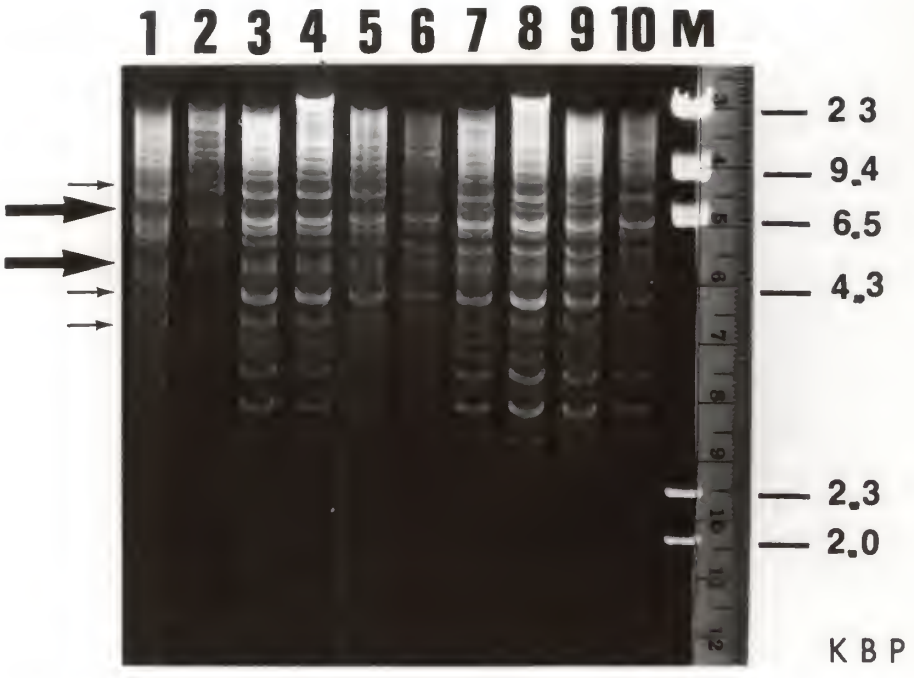

1 2 3 4 5 6 7 8 9 10 M
2 3
9.4
6.5
4.3
2.3
2.0
K B P

Fig. 5. Agarose gel electrophoretic patterns of EcoR I digestion of mt-DNA from S. halepense (1), Redlan B (2), Redlan A (3) and S. versicolor (4). Molecular weight marker, bacteriophage lamda DNA digested by EcoR I (M).

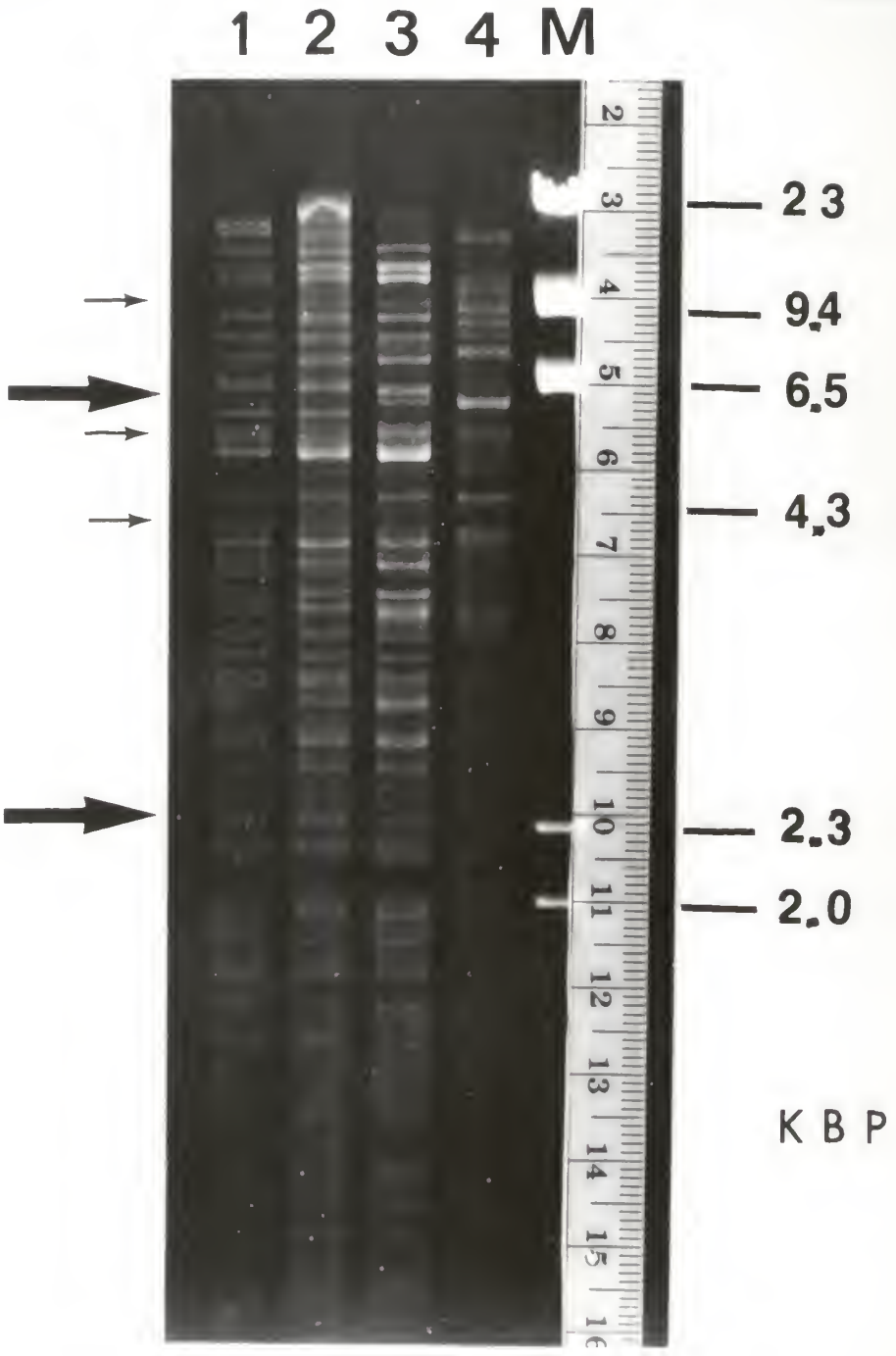
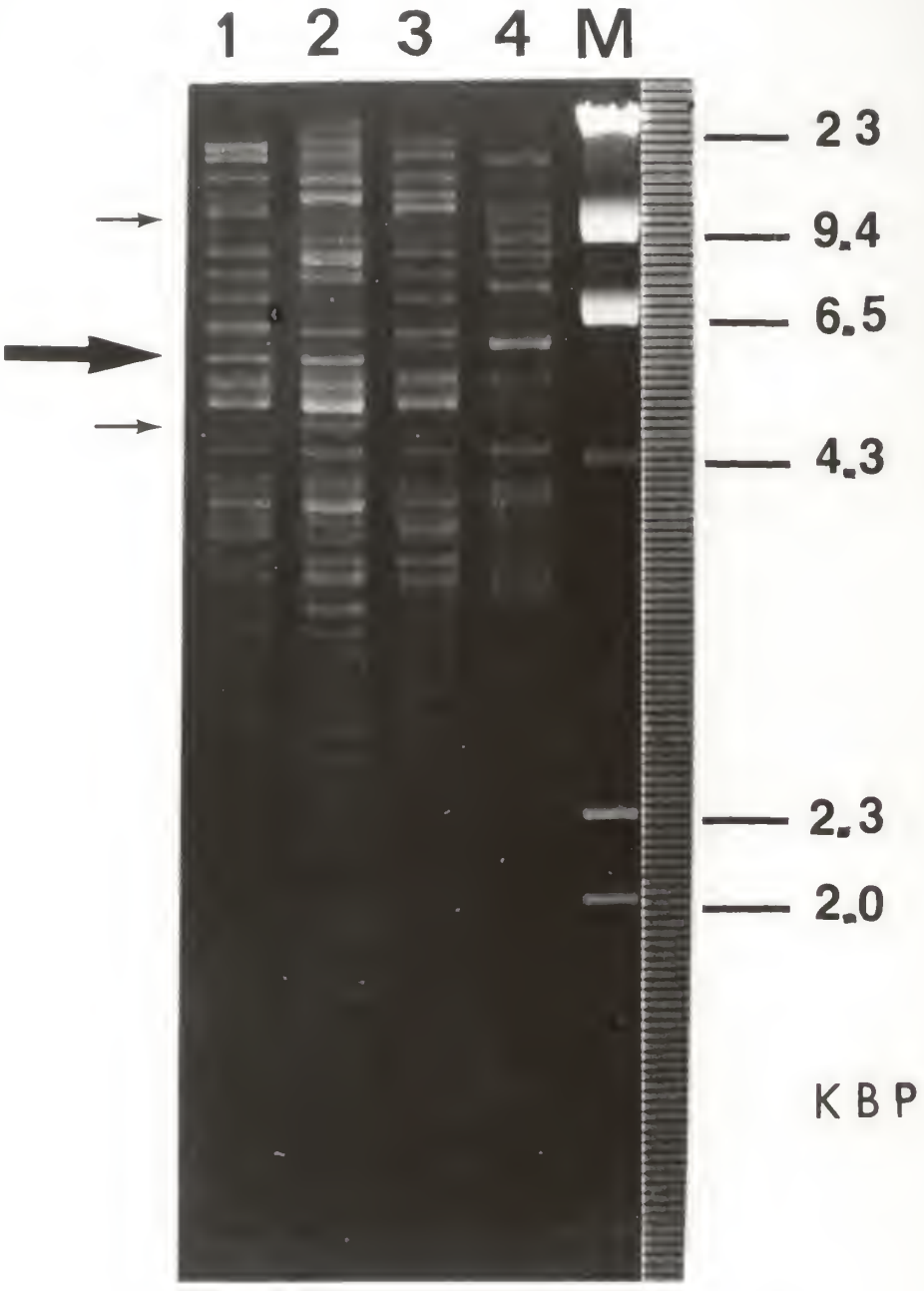

1 2 3 4 M
23
9.4
6.5
4.3
2.3
2.0
K B P

Fig. 6. Agarose gel electrophoretic patterns of EcoR I digestion of mt-DNA from S. halepense (1), KS5 B (2), KS5 A (3) and S. versicolor (4). Molecular weight marker, bacteriophage lamda DNA digested by EcoR I (M).


1
2
3
4
M
23
9.4
6.5
4.3
2.3
2.0
KBP

MITOCHONDRIA DNA RESTRICTION ENDONUCLEASE PATTERNS IN SORGHUM

by

HSING CHUNG LEE

B.A., Tung-Hai University, 1982

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Genetics

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1986

ABSTRACT

Mitochondrial DNAs (mt-DNA) were isolated from Sorghum versicolor (2n=10), S. halepense (2n=40) and 11 male sterile, 5 male fertile lines of S. bicolor. Restriction endonucleases EcoR I, Hind III, and BamH I were used. Restriction patterns were compared by agarose gel electrophoresis.

EcoR I digestion showed that S. versicolor had a unique mt-DNA restriction pattern that was completely different from other sorghum mt-DNA restriction patterns examined. There were some similarities among sorghums that had chromosome numbers 2n=20 and 2n=40. Sumac and KS5 B had distinct fragments in all three digestions, showing that their cytoplasms were different from other lines to a certain degree. S. halepense had almost the exact restriction pattern as S. bicolor. These observations agreed with cytogenetic and morphology observations suggesting that the cytoplasm of S. versicolor was distinctly different from other sorghums examined and the cytoplasm of S. halepense is extremely similar to S. bicolor.

The mt-DNA restriction patterns of cytoplasmic male sterile lines differed, consistently and in the same region, from those of male fertile lines in specific bands depending on the enzymes used. The differences between male sterile and fertile lines are probably attributable to the specific region (or regions) that is related to cytoplasmic male sterility.