Theor Appl Genet (1990) 80:81–87

THEORETICAL AND APPLIED GENETICS

© Springer-Verlag 1990

Mitochondrial DNA variation in long-term tissue cultured rice lines *

M. K. U. Chowdhury [1,], G. W. Schaeffer [2], R. L. Smith [1], L. R. DeBonte [3] and B. F. Matthews [2]**

[1] Agronomy Department, University of Florida, Gainesville, FL 32611, USA
[2] U.S. Department of Agriculture, ARS, Plant Molecular Genetics Laboratory, Beltsville, MD 20705, USA
[3] DNA Plant Technology Corporation, 2611 Branch Pike, Cinnaminson, NJ 08077, USA

Received October 16, 1989; Accepted February 9, 1990
Communicated by P. L. Pfahler

Summary. The effects of long-term tissue culture on mitochondrial DNAs were examined using rice (*Oryza sativa*) cell suspension cultures. Mitochondrial DNAs were isolated from P. I. 353705 (an *indica* subspecies of rice similar to 'Asam 5'), its anther-culture-derived line BL2 (an 8-year-old cell suspension culture), and five other cell lines (A1, A7, A11, A13, and A23), also derived from BL2 and independently selected for resistance to the lysine analog, S-(2-amino)-ethyl-L-cysteine. Mitochondrial DNAs of the rice lines were digested with ten restriction endonucleases (BamHI, BglII, EcoRI, EcoRV, HindIII, PstI, PvuII, SalI, SmaI, and XhoI), electrophoresed, and transferred to nylon membranes. Southern blots were hybridized with one rice and five maize probes containing mitochondrial genes. The restriction patterns of ten Southern blots and hybridization patterns of 60 endonuclease/probe combinations were analyzed. DNAs from all sources produced unique restriction patterns when digested with HindIII or BglII; with the other endonucleases an array of similarities and differences was observed. Lines BL2 and A11 showed unique patterns with all restriction endonucleases tested. No hybridization pattern differences were observed among the lines when probes containing apt9 and atpA were used. However, extensive hybridization pattern differences were observed with coxI, coxII, rrn18-rrn5, and atp6 probes. Both restriction and hybridization patterns revealed variation due to tissue culture effect. CoxII was most efficient in revealing the uniqueness of BL2. Among the analog selected lines A11 was most divergent, and probes rrn18-rrn5 and atp6 were most efficient in revealing its distinctiveness. Unique mitochondrial genomic organizations were found to be associated with long-term tissue culture.

Key words: Rice (*Oryza sativa*) – Mitochondrial DNA – S-(2-amino)-ethyl-L-cysteine – Tissue culture – Restriction and hybridization patterns

* Florida Agricultural Experiment Station Journal Series No. R-00213
** Present address: Vegetable Crops Department, University of Florida, Gainesville, FL 32611, USA

Introduction

The passage of cells through in vitro cell division cycles induces a wide range of variation in many plant species. Several reports have already documented the morphological, biochemical, and chromosomal variation arising from tissue culture (Schaeffer 1982; Armstrong and Phillips 1988). The effect of culture on the nuclear and organelle genomes of plant cells is not well understood. Culture conditions appear to affect the stability of plant genomes, with plant species and genotypes responding differently. Often plants regenerated from undifferentiated cell cultures are not identical and have undergone genetic changes (Armstrong and Phillips 1988). Organelle genomes have shown varying degrees of instability during culture. Chloroplast (ct) and mitochondrial (mt) genomes of carrot were stable over 10 years when maintained in cell suspension culture (Matthews and DeBonte 1985), whereas the mtDNAs of maize showed minor differences after 4 years of culture (McNay et al. 1984). Comparison of mtDNA from wheat embryogenic callus cultures to that of the source parent indicated a novel restriction fragment developed during culture. Some of the restriction fragments underwent quantitative changes (amplification, decrease, or loss) and the variation was rapidly stabilized following the first subculture (Hartmann et al. 1989). A recent report strongly suggests that,

in wheat callus cultures, a particular mitochondrial genome organization is correlated with the ability of cultured cells to regenerate whole plants (Rode et al. 1988).

Extensive variation was observed in rice plants recovered from either anther (Schaeffer 1982) or tissue culture (Oono 1978; Schaeffer and Sharpe 1987). Schaeffer and Sharpe (1987) found that self-fertilized rice plants regenerated from callus that had been exposed to high (1 or 2 m*M*) lysine and threonine produced progeny containing chlorophyll and floral morphology mutants and plants varying in seed lysine content. Cell suspension lines resistant to S-AEC and from nonselected callus have been maintained for more than 8 years. Those cell lines were routinely subcultured every 4–6 weeks and have stabilized into uniform color, aggregation pattern, and growth rates.

In this paper we describe the mtDNA variation found in six long-term, cell suspension rice lines, mentioned above, and the source parent plant. Five of the cultured lines were selected for S-AEC resistance and one was not. The objectives of these experiments were to evaluate and characterize the mtDNA variation generated during long-term rice cell cultures using endonuclease restriction fragment and hybridization patterns of known organelle gene probes.

Materials and methods

Rice materials

Rice materials used in this investigation were P. I. 353705, an *indica* subspecies line similar to Assam 5, designated as A5; BL2, a doubled haploid cell suspension line originating as callus from culture of A5 anthers (Schaeffer and Sharpe 1981), then maintained in cell suspension for 8 years; and five cell lines from BL2 selected for resistance to the lysine analog, S-(2-amino)-ethyl-L-cysteine (S-AEC). For those selections, callus tissue was subjected to three passages on 1 m*M* S-AEC and one final passage on 2 m*M* S-AEC (Schaeffer and Sharpe 1981), and those cultures have been maintained for 8 years in cell suspension. The S-AEC resistant lines are designated as A1, A7, A11, A13, and A23.

Mitochondrial DNA isolation

Mitochondrial DNA was isolated from 10-day-old etiolated seedlings of A5 using the method as described by Smith et al. (1987). In summary, etiolated seedling tissues were ground in a Waring blender using a saline extraction buffer, followed by differential centrifugation to isolate mitochondria and by DNAse treatment to remove extramitochondrial DNA. Mitochondrial DNAs were purified by precipitating SDS-protein-carbohydrate complexes with potassium acetate, precipitating mtDNA with isopropanol followed by phenol, phenol-chloroform, and chloroform extraction and a second isopropanol precipitation.

MtDNAs were isolated from cell suspension cultures of BL2 and S-AEC resistant lines using the method as described by DeBonte and Matthews (1984). In this method, cell suspensions were ground in liquid N_2 and extracted with a buffer containing sorbitol as an osmoticum. Mitochondria were isolated by differential centrifugation, lysed with N-lauryl sarcosine, and mtDNA was purified using CsCl-bisbenzamide gradient centrifugation.

Restriction endonuclease analysis

Restriction endonuclease digestions of the mtDNAs were conducted using ten enzymes according to the enzyme supplier's instructions. DNA fragments were separated by gel electrophoresis in 0.8% agarose in TPE buffer (0.08 *M* *TRIS*-phosphate and 2 m*M* EDTA, pH 8.0). Gels were run at room temperature for 16 h at 2 V/cm. Gels were stained with 0.5 $\mu g\ ml^{-1}$ ethidium bromide for 45 min and photographed using standard procedures. Numbers of variable fragments were counted for pairwise comparisons and sizes were calculated. HindIII-digested lambda DNA alone or mixed with HaeIII-digested Phi × 174 DNA molecular size markers were used.

Isolation and preparation of probes

Maize clones containing mitochondrial genes cytochrome c oxidase subunit I (coxI) (Isaac et al. 1985), subunit II (coxII) (Fox and Leaver 1981); $F_1 - F_0$ ATPase subunit 6 (atp6) (Dewey et al. 1985a), subunit 9 (atp9) (Dewey et al. 1985b), subunit alpha (atpA) (Braun and Levings 1985), and 18S – 5S ribosomal RNAs (rrn18-rrn5) (Chao et al. 1984) were provided by C. S. Levings, III, of North Carolina State University, Raleigh/NC, USA. The rice clone containing coxII gene (Kao et al. 1984) was provided by R. Wu of Cornell University, Ithaca/NY, USA.

Probes consisted of cloned maize and rice DNA without the vector. Maize and rice inserts were recovered following electrophoresis using the NA45 membrane (Schleicher and Schuell, Inc., Keene/NH) according to the supplier's directions. The NA45 method involved using electrophoresis in regular agarose, placing the membrane in a slit in the gel directly below the fragment of interest, electrophoresing the fragment onto the strip of NA45 membrane, then eluting the membrane-bound DNA. The maize coxII gene probe was used only for preliminary studies. For subsequent studies, a probe (E2) containing a 0.6-kb SalI-HindIII fragment from the second exon of the rice coxII (Kao et al. 1984) was used. The DNA probes were labelled with ^{32}P by nick-translation as described by Rigby et al. (1977). Unincorporated nucleotides were removed by chromatography through sephadex G-50.

Southern transfers and hybridization of DNA

DNA was transferred to Nytran membrane (Schleicher and Schuell, Inc., Keene/NH) according to Southern (1975). Prehybridization was carried out at 65 °C for 2 h using 10 × Denhardt's solution (1 × = 0.1% SDS, 0.2% PVP-360, 0.2% Ficol-400, and 0.2% BSA), 3 × SSC (1 × = 0.14 *M* NaCl and 0.015 *M* sodium acetate), and 5 $mg\ ml^{-1}$ denatured salmon sperm DNA. For a 20 × 20 cm blot, 30 ml prehybridization solution containing 0.5 ml salmon sperm DNA was used. Hybridization was carried out for 16 h in fresh prehybridization mixture with the boiled probe added. Following hybridization, membranes were washed twice in 3 × SSC and once in 0.3 × SSC at 65 °C, then autoradiographed.

Results

Mitochondrial restriction patterns

Mitochondrial DNAs from all sources produced unique restriction fragment patterns when digested with HindIII (Fig. 1 A) or BglII; with other enzymes (BamHI, shown in Fig. 1 B), an array of similarities and differences were observed (Table 1). BL2 and A11 showed unique patterns

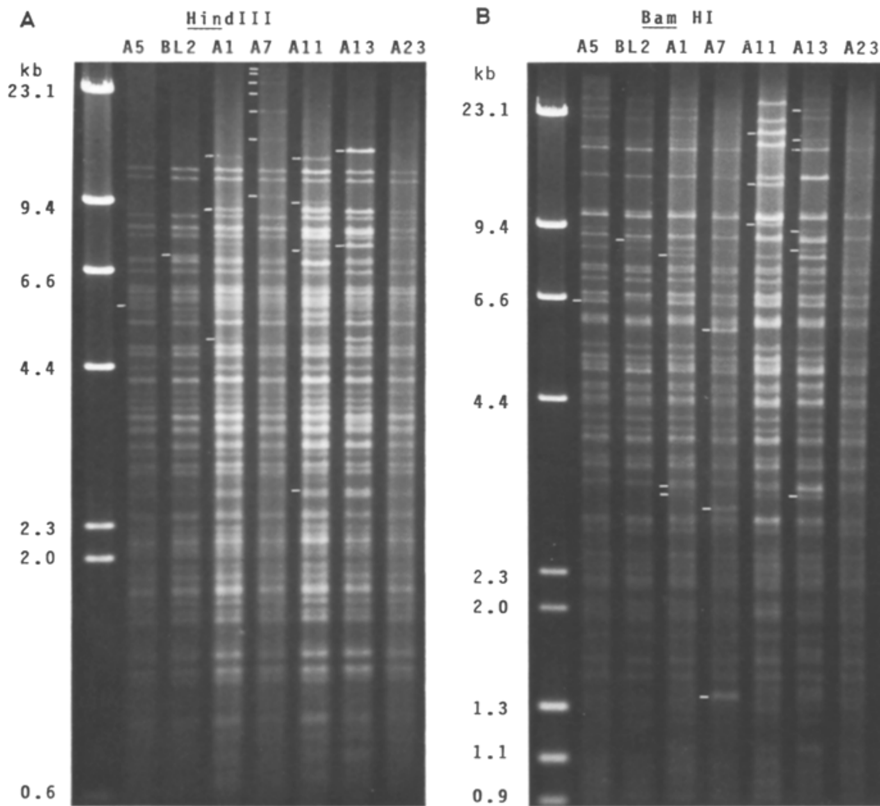

Fig. 1. **A** Restriction patterns of mtDNAs from A5, BL2, A1, A7, A11, A13, and A23 generated by HindIII. *Left lane* contains the HindIII-digested lambda marker DNA and the sizes of those marker fragments are shown in kb. **B** Restriction patterns of mtDNAs of the above rice lines generated by BamHI. *Left lane* contains the HindIII-digested lambda and HaeIII-digested Phi × DNA, and the sizes of those marker fragments are shown in kb. *Dashes* (−) indicate the presence of variable fragments in the rice genotypes

Table 1. Restriction endonuclease fragment pattern summary of A5, BL2, A1, A7, A11, A13, and A23 mtDNAs digested with ten enzymes. Rice lines connected together with underlining have similar restriction patterns

Enzyme	Pattern						
HindIII	A5	BL2	A1	A7	A11	A13	A23
BglII	A5	BL2	A1	A7	A11	A13	A23
EcoIV	A5	BL2	A1	A13	A7	A11	A23
EcoRI	A5	BL2	A1	A23	A7	A11	A13
BamHI	A5	A23	BL2	A1	A13	A7	A11
SalI	A5	A1	BL2	A7	A23	A11	A13
PvuII	A5	A7	BL2	A1	A13	A23	A11
PstI	A5	A1	A7	A23	A13	BL2	A11
XhoI	A5	A1	A7	A23	A13	BL2	A11
SmaI	A5	A7	A13	A23	BL2	A1	A11

with every restriction endonuclease tested. PstI and XhoI had the least resolving power and revealed identical restriction patterns for A5, A1, A7, A13, and A23. PvuII, PstI, XhoI, and SmaI revealed the similarity between A5 and A7. No consistent similarity was observed among the restriction patterns of the S-AEC-resistant cell lines. When comparisons were made to A5, the primary source of all cultures, maximum variability was observed in the HindIII-digested mtDNA, when all the variable fragments were pooled, and minimum variability was observed in the XhoI-digested mtDNA (Figs. 1 and 2). When the S-AEC-selected lines were compared to BL2, most variation was observed with HindIII-digested mtDNA and least variation with PvuII-digested mtDNA (Figs. 1 and 2). HindIII-digested mtDNA revealed a number of unique fragments in the lines used, e.g., a 7.4-kb fragment in BL2, 30.0-, 20.0-, 15.7-, and 10.5-kb fragments in A7, a 5.4-kb fragment in A11, and a 15.0-kb fragment in A13.

All the lines had a 5.7-kb fragment except BL2. Of the eight fragments found in A11, A23 had only the 9.8-kb fragment and A1 had all the fragments except the 5.4-kb fragment. Similarly, BL2 revealed a unique fragment of 9.0 kb, A7 revealed unique fragments of 5.9, 2.8, and 1.4 kb, and A11 had unique fragments of 19.7, 13.1, and 10.1 kb when digested with BamHI. The 6.6-kb fragment present in all of the S-AEC-resistant lines was absent in BL2. A1 and A13 had the same pattern. A5 and A23 also had the same pattern. A1 had two extra fragments of 8.6 and 3.0 kb not found in A5. To avoid a lengthy pattern description for each endonuclease, the patterns of the other eight enzymes are presented as a schematic diagram in Fig. 2A and B. Unique patterns of common bands among A1, A11, A13, and A23 were observed when digested with BglII, EcoRI, and EcoRV.

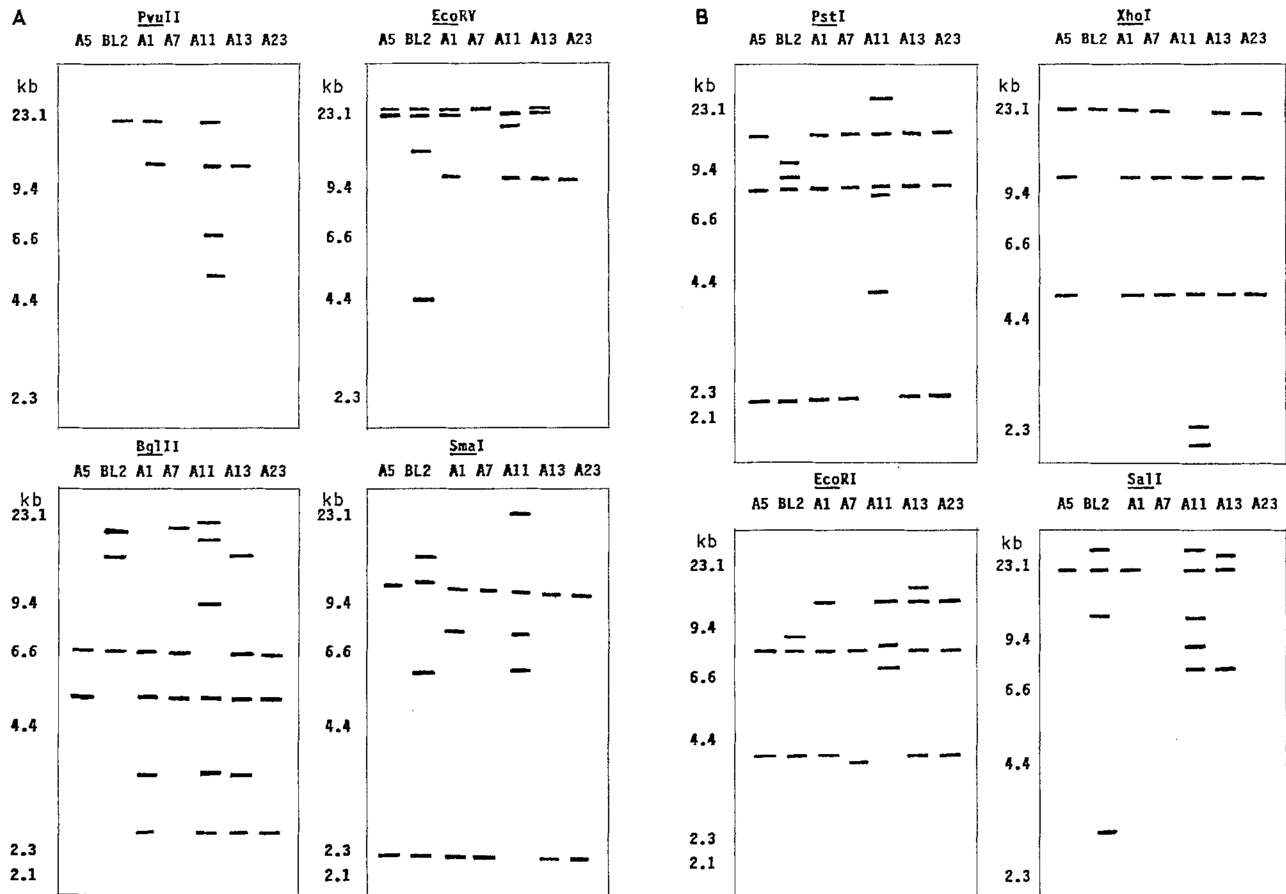

Fig. 2A and B. Restriction patterns of variable fragments of mtDNAs from the rice lines A5, BL2, A1, A7, A11, A13, and A23. **A** digested with PvuII, EcoRV, BglII, and SmaI, and **B** digested with PstI, XhoI, EcoRI, and SmaI are shown. Sizes (in kb) of the markers described in Fig. 1 are listed on the *left*

Hybridization patterns

The coxII maize gene, used to probe a SalI digest of the seven lines, hybridized strongly to a 2.0-kb fragment present in all the lines, to a 2.3-kb fragment present only in BL2, and weakly to a number of other fragments. The rice coxII gene has a 5′ exon (E1), an intron containing a BamHI site, and a 3′ exon (E2). In order to find out what part of the gene hybridized to the unique BL2 fragment, the probe was digested with BamHI and the fragments containing the E1 and E2 sequences were separately hybridized to SalI-digested blots. Only the probe containing the E2 sequence had homology with the 2.3-kb BL2 fragment, indicating the presence of sequences similar to the coxII 3′ exon in BL2. For subsequent coxII hybridizations, a 0.6-kb (SalI-HindIII) piece of mtDNA containing the E2 of the rice clone (Kao et al. 1984) was used.

Hybridization patterns of the coxI, coxII, rrn18-rrn5, and atp6 probes hybridized to Southern transfers of rice mtDNA digested with XhoI, BglII, BamHI, HindIII, and SmaI and are shown in Fig. 3. Maize coxI sequence hybridized to XhoI-digested DNA of the seven lines revealed a unique 16.5-kb fragment in A11, a 21.8-kb fragment in A11 and A13 (strong signal) and in A1 (weak signal) (Fig. 3A). The coxI probe when hybridized to BglII-digested blot detected a unique fragment of 1.6 kb in BL2 and a fragment of 3.0 kb in A1, A11, and A13 (Fig. 3A). The rice coxII probe hybridized to unique BamHI fragments of 4.6 kb in BL2 and 16.7 kb in A11, and to a 9.5-kb BamHI fragment present in all the lines except A11, where it was missing (Fig. 3A).

The rice coxII probe hybridized to three HindIII fragments, two of which, 8.1 and 3.7 kb, were present in all lines, and a 5.7 kb fragment that was present in all the lines except BL2 (Fig. 3A). The rrn18-rrn5 probe hybridized unique BglII fragments of 10.2 kb in BL2, and of 3.8 kb in A1, A11, A13, and A23. Two BglII fragments of 28.9 and 12.0 kb were present in all the lines except A11 (Fig. 3B). The rrn18-rrn5 probe hybridized to five HindIII fragments that were common to all the lines, and unique fragments of 1.6 kb in A11 and 2.4 kb in A1, A11, A13, and A23 (Fig. 3B). The atp6 probe hybridized to unique BamHI fragments of 8.5 kb in BL2, of 31.2 and

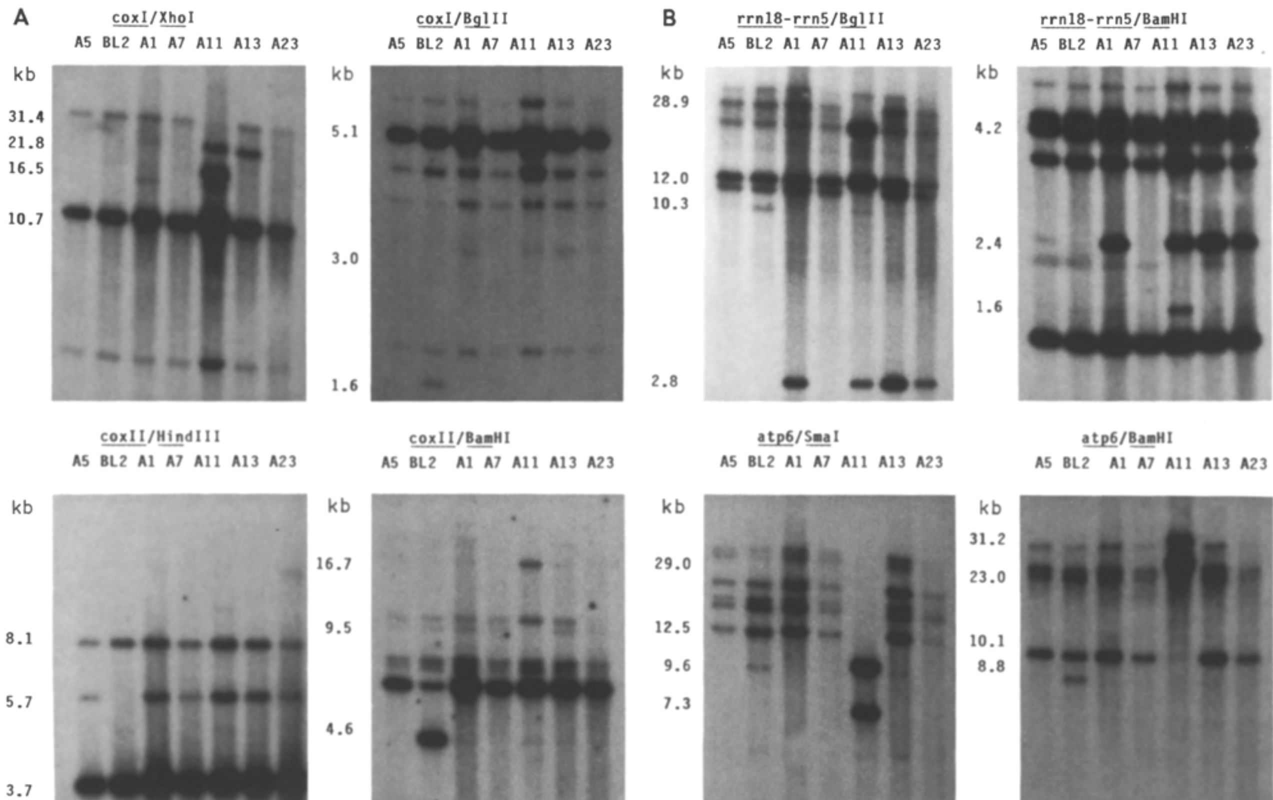

Fig. 3 A and B. Hybridization patterns of mtDNAs of A5, BL2, A1, A7, A11, A13, and A23 are shown. **A** coxI and coxII pattern, and **B** rrn18-rrn5 and atp6 pattern. Numbers to the *left* of each autoradiograph indicate the sizes (in kb) of the hybridized fragments

Table 2. Hybridization pattern summary of A5(5), BL2(B), A1(1), A7(7), A11(11), A13(13), and A23(23) mtDNAs digested with ten enzymes and hybridized with six probes. Rice lines connected together with underlining have similar hybridization patterns. ND = No difference, and NDR = No differences among rest of the lines

Enzyme	Probe				
	CoxI	CoxII	rrn18-rrn5	atp6	atp9	atpA
HindIII	B 7 NDR	B NDR	ND	11 NDR	ND	ND
BglII	B 5 7 23 1 11 13	B NDR	5 7 B 11 1 13 23	11 NDR	ND	ND
EcoRV	5 B 7 11 1 13 23	B NDR	11 NDR	B 11 NDR	ND	ND
EcoRI	5 B 7 NDR	B NDR	5 B 7 13 1 11 23	ND	ND	ND
BamHI	1 B 5 7 11 13 23	B NDR	5 B 7 11 1 13 23	B 11 NDR	ND	ND
SalI	ND	B A11 NDR	B 11 NDR	A11 NDR	ND	ND
PvuII	5 B 7 23 1 11 13	B NDR	11 NDR	B A11 NDR	ND	ND
PstI	11 13 NDR	B NDR	5 B 7 11 1 23 13	ND	ND	ND
XhoI	11 13 NDR	B 11 NDR	5 B 7 11 1 13 23	ND	ND	ND
SmaI	ND	B 11 NDR	11 NDR	B 11 NDR	ND	ND

23.0 kb in A11 (Fig. 3B). Three fragments of sizes 27.4, 19.0, and 10.0 kb were present in all the lines except A11 (Fig. 3B). The atp6 probe hybridized to six SmaI fragments ranging in size from 12.5 to 29.0 kb in all lines except A11. However, it hybridized to a unique SmaI fragment of 7.3 kb present only in A11, and a 9.6 kb fragment present in A11 and BL2 (Fig. 3B).

No hybridization pattern differences among the lines for any of the enzymes used were observed when atp9 and atpA were used as probes. However, hybridization pattern differences were observed with coxI, coxII, rrn18-rrn5, and atp6 with most of the endonuclease digestions used (Table 2). A5, BL2, and A7 showed similar hybridization patterns in 16 of the 20 enzyme-probe combinations when tested with coxI and rrn18-rrn5 probes. On the other hand, the uniqueness of the BL2 line was revealed by coxII with all endonucleases tested. The uniqueness of the A11 line was revealed by 22 of the 40 enzyme-probe combinations tested. The rrn18-rrn5 and atp6 probes were most efficient in detecting the uniqueness of A11.

Discussion

All seven lines used in this investigation have unique mtDNA restriction patterns. MtDNA rearrangements were observed when the tissue-cultured (BL2) mtDNA was compared to the non-tissue-cultured line (A5) and when the mtDNA of the S-AEC-selected lines (A1, A7, A11, A13, and A23) was compared to that of either BL2 or A5. These mtDNA variations or rearrangements may have arisen from a number of sources: (1) there may be a heterogeneous population of mitochondria present in A5, the primary source of all these cell lines; (2) the culturing process itself may induce changes in the mtDNA through deletion, duplication, translocation, and/or base pair change (point mutation); (3) the S-AEC treatment may have induced mtDNA changes; and (4) the S-AEC treatment may have allowed certain variant cells to divide more rapidly and become the predominant cells in a culture.

The presence of heterogeneity in the A5 mt population is the least probable source of variation because mtDNAs isolated from a number of batches of A5 seeds were not variable (data not presented). A similar result was reported with maize by Gengenbach et al. (1981). The culturing process has been reported to induce mtDNA rearrangement or variation (Chowdhury et al. 1988; DeBuyser et al. 1988; Hartmann et al. 1989). Kemble and Shepard (1984) suggested that culturing higher plant tissue may be regarded as a process that increases the molecular diversity of the mt genome.

In these experiments, mt genomic changes were observed in every one of the six culture lines studied. The BL2 data show that long-term culture can stimulate mt genomic rearrangements. However, one might question whether long-term tissue culture alone was responsible for the extensive mt changes observed in the six lines or whether selection with S-AEC may have played an important role by causing certain variant cell types to predominate in culture. Our results show considerable variation exists between the S-AEC selected and nonselected rice lines, which is similar to other reports (Gengenbach et al. 1981; Kemble and Shepard 1984). The high level of mtDNA rearrangements noted in several of the selected lines (number of variable fragments counted from Figs. 1 and 2) may lead one to suspect that the process of selection for S-AEC resistance may have caused changes or selected cells with mt rearrangements. However, when the numbers of variable fragments in all of the S-AEC-selected rice lines are considered, a near normal distribution of variation is observed around BL2.

When coxII was used as a probe, BL2 had a hybridization pattern different from the rest of the lines with all ten enzymes used. Although both the BL2 and the S-AEC-selected lines were in cell suspension about the same length of time, the unique BL2 pattern was not present in any of the S-AEC-selected lines, which may indicate that the unique change in the coxII region of the BL2 occurred after the S-AEC selection and, therefore, is not a selection effect but a tissue culture effect, and also that an association of tissue culture with mtDNA rearrangement exists. The rrn18-rrn5 gene probe hybridized to the unique 2.8-kb BglII and 2.4-kb BamHI fragments present in all the selected lines, except in A7. Restriction pattern data also show that A7 is different from the other S-AEC-selected lines and similar to the nonselected lines, A5 and/or BL2.

This research has established that extensive mt genomic variation was generated by long-term tissue culture. The data leads one to question whether selection with S-AEC also affects mt genomic stability. However, separation of the effects of long-term tissue culture from those of the S-AEC treatments in these materials would require the analysis of mtDNA isolated from both the BL2 callus and the S-AEC-selected callus tissue immediately after the selection treatment. Since those tissues are not available, we cannot distinguish effects of S-AEC treatment from those of tissue culture. Future research to evaluate the effects of S-AEC selection on mt genomic stability would require that the S-AEC selection treatments be redone with mtDNA evaluations following immediately.

References

Armstrong CL, Phillips RL (1988) Genetic and cytogenetic variation in plants regenerated from organogenic and friable, embryogenic tissue cultures of maize. Crop Sci 28:363–369

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 (1984) Nucleotide sequence and evolution of the 18S ribosomal RNA gene in maize mitochondria. Nucleic Acids Res 12:6629–6644

Chowdhury MKU, Schaeffer GW, Smith RL, Matthews BF (1988) Molecular analysis of organelle DNA of different subspecies of rice and the genomic stability of mtDNA in tissue cultured cells of rice. Theor Appl Genet 76:533–539

DeBonte LR, Matthews BF (1984) Rapid isolation and purification of plastid and mitochondrial DNA from carrot cell suspensions. Plant Mol Biol Rep 2:32–36

DeBuyser J, Hartmann C, Henry Y, Rode A (1988) Variations in long-term wheat somatic tissue culture. Can J Bot 66:1891–1895

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

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

Gengenbach BG, Connelly JA, Pring DR, Conde MF (1981) Mitochondrial DNA variation in maize plants regenerated during tissue culture selection. Theor Appl Genet 59:161–167

Hartmann C, Henry Y, DeBuyser J, Aubry C, Rode A (1989) Identification of new mitochondrial genome organizations in wheat plants regenerated from somatic tissue cultures. Theor Appl Genet 77:169–175

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

Kao T, Moon E, Wu R (1984) Cytochrome oxidase subunit II gene of rice has an insertion sequence within the intron. Nucleic Acids Res 12:7306–7315

Kemble RJ, Shepard JF (1984) Cytoplasmic DNA variation in a potato protoclonal population. Theor Appl Genet 69:211–216

Matthews BF, DeBonte LR (1985) Chloroplast and mitochondrial DNAs of carrot and its wild relatives. Plant Mol Biol Rep 3:12–14

McNay JW, Chourey PS, Pring DR (1984) Molecular analysis of genomic stability of mitochondrial DNA in tissue cultured cells of maize. Theor Appl Genet 67:433–437

Oono K (1978) High frequency mutations in rice plants regenerated from seed callus. In: Proc 4th Int Congr Plant Tiss Cell Cult, Calgary, Canada. p 52

Rigby PWJ, Dieckmann M, Rhodes C, Berg P (1977) Labeling deoxynucleic acids to high specific activity in vitro by nick translation with DNA polymerase I. J Mol Biol 113:237–251

Rode A, Hartmann C, DeBuyser J, Henry Y (1988) Evidence for a direct relationship between mitochondrial genome organization and regeneration ability in hexaploid wheat somatic tissue cultures. Curr Genet 14:387–394

Schaeffer GW (1982) Recovery of heritable variability in anther-derived doubled haploid rice. Crop Sci 22:1160–1164

Schaeffer GW, Sharpe FT (1981) Lysine in seed protein from S-aminoethyl-L-cysteine resistant anther-derived tissue cultures of rice. In Vitro 17:345–352

Schaeffer GW, Sharpe FT (1987) Increased lysine and seed storage protein in rice plants recovered from calli selected with inhibitory levels of lysine plus threonine and S-(2-aminoethyl) cysteine. Plant Physiol 84:509–515

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

Southern EM (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 98:503–517