Theor Appl Genet (1989) 77:617–619

Small mitochondrial DNA molecules of wild abortive cytoplasm in rice are not necessarily associated with CMS

N.M. Saleh, B.J. Mulligan, E.C. Cocking and H.S. Gupta*

Plant Genetic Manipulation Group, Department of Botany, University of Nottingham, Nottingham NG7 2RD, UK

Received November 15, 1988; Accepted January 9, 1989
Communicated by G.S. Khush

Summary. Mitochondrial DNA was isolated from leaf tissue of both the cytoplasmic male sterile line of *Indica* rice variety V41, which carries wild abortive (WA) cytoplasm, and from the corresponding maintainer line. In addition to the main mitochondrial DNA, four small plasmid-like DNA molecules were detected in both the male sterile and fertile lines. Restriction analysis of total mitochondrial DNA from the male sterile and fertile lines showed DNA fragments unique to each. Our findings suggest that the four small mitochondrial DNA (mtDNA) molecules are conserved when WA cytoplasm is transferred into different nuclear backgrounds. However, there is no simple correlation between the presence/absence of small mitochondrial DNA molecules and the expression of WA cytoplasmic male sterility (CMS).

Key words: Cytoplasmic male sterility – Mitochondrial DNA – Plasmid-like molecules – Wild abortive cytoplasm – Rice

Introduction

Cytoplasmic male sterility (CMS) is a failure to produce or release pollen, due to an incompatibility between nuclear and mitochondrial genes. For commercial production of hybrid seed, CMS lines are crossed with varieties containing dominant nuclear genes for restoration of fertility. CMS is maternally inherited and is controlled by mitochondrial genes (Laughnan and Gabay-Laughnan 1983). Maintenance of a particular CMS line requires it to be crossed with a fertile maintainer carrying the same nuclear genome. However, the cytoplasm of the maintainer is not necessarily related to that of the corresponding CMS line. CMS in rice has been classified into four cytoplasmic types: S_1 (originally Chinsurah Boro II), S_2 (derived from wild abortive or WA), S_3 (Gambiaca) and S_4 (not designated earlier) (Virmani et al. 1986; Young et al. 1983).

Molecular analysis of mtDNA from maize (Kemble and Bedbrook 1980), sorghum (Dixon and Leaver 1982), sugar beet (Powling 1981) and rice (Yamaguchi and Kakiuchi 1983; Kadowaki et al. 1986; Mignouna et al. 1987; Shikanai et al. 1987) indicated differences in the occurrence of small linear or circular DNA molecules in CMS and male fertile lines. In rice, mitochondria of S_1 CMS lines have been shown to possess two (Yamaguchi and Kakiuchi 1983) or four (Kadowaki et al. 1986) plasmid-like molecules in addition to the main mtDNA. In contrast, four species of low molecular weight plasmid-like DNA have been identified in mitochondria associated with S_2 CMS (Mignouna et al. 1987). In the latter investigation, WA cytoplasm was present in the nuclear background of Zhen Shan 97, a Chinese *Indica* rice. In the present report, the similar occurrence of four low molecular weight mtDNA molecules in the WA cytoplasm (S_2 CMS) associated with a different nuclear genome (*Indica* variety V41) is described. This finding suggests that WA CMS transfer into different nuclear backgrounds does not markedly influence the organization of low molecular weight mitochondrial DNA molecules. In addition, both CMS and maintainer lines of V41 contain the same four small mtDNA molecules. Thus, WA CMS is not necessarily associated with the presence or absence of plasmid-like mtDNA.

* Present address: Division of Plant Breeding, ICAR Research Complex for North Eastern Hills Region, Shillong – 793004, India

Materials and methods

Plant material

The *Indica* rice CMS line V41A was developed from a wild rice, *Oryza sativa* f. *spontanea* L., carrying wild abortive (WA) cytoplasm (Lin and Yuan 1980). The nuclear source was the Chinese rice variety V41. Seeds of the male sterile (V41A) and fertile maintainer (V41B) lines were supplied by Dr. S. S. Virmani, International Rice Research Institute (IRRI), Manila, the Philippines.

Isolation of mitochondrial DNA

Seeds were dehusked and surface-sterilized with 30% 'Domestos' bleach (Lever Brothers, UK) for 45 min and washed with 6 changes of sterile water. Seeds were sown on hormone-free agar-solidified (0.8% w/v; Sigma) MSO medium (Murashige and Skoog 1962) and maintained under continuous light (2,000 lux, Thorn 36 W. Pluslux 3500) at 28 ± 2 °C. Fourteen-day-old seedlings were placed in the dark for 3 days prior to isolation of mtDNA.

The method used for mtDNA extraction was a modification of a published procedure (Kemble 1987). All procedures were performed at 4 °C, unless otherwise stated. Fifteen to twenty grams of leaves from 14-day-old seedlings were harvested and cut into small pieces on a chilled tile. The tissue was then homogenized with a mortar and pestle in 100 ml of Buffer A (10 m*M* TES pH 7.2, 0.5 *M* Mannitol, 0.2% BSA and 0.05% cysteine). The homogenate was filtered through four layers of cheese cloth and one layer of Miracloth (Calbiochem), and centrifuged at 5,000 rpm for 10 min (JA20 rotor) in a Beckman J2-21 centrifuge. The supernatant was collected. The pellet was gently resuspended in 30 ml of Buffer A using a fine brush and recentrifuged at 5,000 rpm for 10 min. Both supernatants were combined and centrifuged at 12,000 rpm for 10 min to concentrate the mitochondria. The supernatant was recentrifuged (12,000 rpm for 10 min). The pooled mitochondrial pellet was resuspended in 10 ml of Buffer A and centrifuged at 5,000 rpm for 10 min. The supernatant containing mitochondria was checked optically for any contamination by chloroplasts and nuclei. Additions of 1 *M* $MgCl_2$ and 10 mg/ml freshly prepared DNase I (in 0.15 *M* NaCl and 50% glycerol) were made to the mitochondrial preparation to give final concentrations of 10 m*M* $MgCl_2$ and 10 µg DNase I/g fresh weight of leaf tissue, respectively. After incubation for 1 h at 4 °C, 2 ml aliquots of mitochondrial suspension were layered onto 6 ml Buffer B (10 m*M* TES pH 7.2, 20 m*M* EDTA in 0.6 *M* sucrose) and centrifuged at 16,000 rpm for 10 min in a Beckman SW41 rotor. The pellet was resuspended in 4 ml Buffer C (50 m*M* TRIS-HCl pH 8.0, 10 m*M* EDTA, 2% sarkosyl and 100 µg/ml Proteinase K) and incubated at 37 °C on a shaker with gentle agitation for 2 h. The lysate was made up to 0.2 *M* ammonium acetate, and the mtDNA was purified by three cycles of phenol-chloroform extraction at room temperature prior to ethanol (70% v/v) precipitation at −20 °C for 24 h. The precipitate was recovered by centrifugation (17,000 × g for 30 min), washed twice with 80% v/v ethanol and resuspended in TE buffer (1 m*M* EDTA, 10 m*M* TRIS, pH 8.0). RNA contamination was reduced by treatment with 100 µg/ml RNase A for 1 h at 37 °C immediately prior to loading samples on gels. mtDNA samples were stored at −20 °C.

Electrophoretic analysis of native mtDNA

The mtDNA was electrophoresed 12–16 h at 25 V in 0.8% agarose gel containing TBE buffer (0.089 *M* TRIS-borate, 0.089 *M* boric acid, 2.5 m*M* EDTA-Na_2). The gel was stained for 30 min in 1.5 µg/ml ethidium bromide in electrophoresis buffer. Molecular weight markers were provided by a HindIII digest of phage lambda DNA.

Results and discussion

Native mtDNA from the male sterile *Indica* rice line V41, containing the WA cytoplasm (S_2 CMS), and from the male fertile maintainer V41B were electrophoresed on 0.8% agarose. Both preparations showed one broad band representing the high molecular weight mitochondrial genome. In addition, both mtDNA preparations possessed four discrete plasmid-like molecules of 2.3, 1.6, 1.5 and 1.2 kb in size, as judged from their mobilities relative to the linear marker DNA bands (Fig. 1). The BamHI restriction profiles of both mtDNA preparations were very similar, although several fragments unique to either V41A or V41B were observed (Fig. 2).

The four low molecular weight mtDNA molecules of V41A and V41B showed similar electrophoretic mobilities to the open circular forms of plasmid-like mtDNA previously detected in WA cytoplasm of variety Zhen Shan 97A (Mignouna et al. 1987). The largest of these species was absent from the corresponding fertile maintainer, Zhen Shan 97B, carrying "normal" fertile cytoplasm. In *Japonica* rice, CMS of the BT type (Chinsurah Boro II, S_1 CMS) appeared to be correlated with differences in mtDNA organization of CMS and fertile lines (Yamaguchi and Kakiuchi 1983; Kadowaki et al. 1986; Shikanai et al. 1987). One plasmid-like mtDNA molecule of 2.1 kb, termed B_1, was partially homologous with the 1.9-kb plasmid of maize mitochondria and was specific to BT-CMS in A58 rice variety (Shikanai et al. 1987).

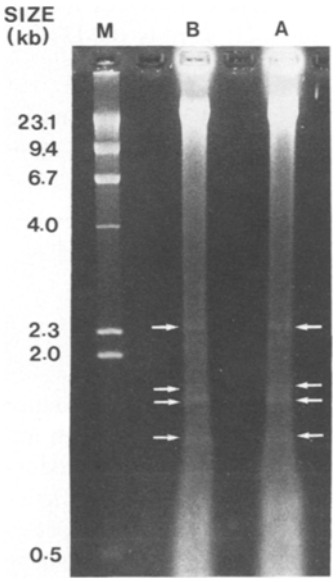

Fig. 1. Agarose gel electrophoretic patterns of total mitochondrial DNA from (*A*) CMS and (*B*) fertile maintainer lines of *Indica* rice variety V41. *Arrows* indicate the location of plasmid-like mitochondrial DNA molecules. Molecular size markers (*M*) are given by a HindIII digest of phage lambda DNA

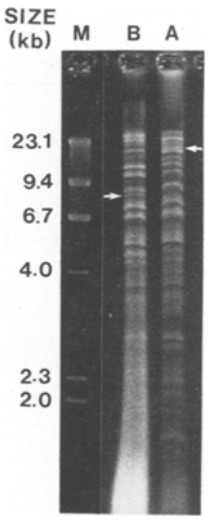

Fig. 2. Agarose gel electrophoretic patterns of BamHI-digested total mitochondrial DNA from (*A*) CMS and (*B*) fertile maintainer lines of *Indica* rice variety V41. *Arrows* indicate fragments unique to V41A and V41B respectively. Molecular size markers (*M*) are given by a HindIII digest of phage lambda DNA

The results presented in the present paper (Fig. 1), however, suggest that there is no clear correlation between the presence and absence of specific low molecular weight mtDNA molecules and WA (S_2) CMS.

Mignouna et al. (1987) identified several differences in the restriction patterns of total mtDNA from Zhen Shan 97A and 97B, which probably represented mutations and/or rearrangements of the high molecular weight mtDNA. The same situation was observed in the BamHI restriction profiles of V41A and V41B mtDNA (Fig. 2). Rottmann et al. (1987) have demonstrated specific alterations (deletions) in mtDNA which accompany the reversion of CMS-T maize to fertility. Whether such events underlie the differences between mtDNA of the CMS line V41A and the fertile maintainer V41B and, thus, distinguish fertile from WA cytoplasm in rice remains to be determined.

Acknowledgements. We thank Dr. S. S. Virmani and Dr. T. T. Chang, IRRI, Los Banos, Laguna, The Philippines, for supplying seeds and information on the origin of rice breeding lines, and T. M. Fenning, J. Chartier-Hollis and M. McLellan for helpful advice. We are grateful to Prof. Y. Nerkar, Marathwada Agricultural University, Parbhani, India, for discussion on CMS in plant breeding. This work was supported in part by the Overseas Development Administration and the Rockefeller Foundation. H.S.G. was a Commonwealth Post-Doctoral Fellow during the course of this investigation.

References

Dixon LK, Leaver CJ (1982) Mitochondrial gene expression and cytoplasmic male sterility in sorghum. Plant Mol Biol 1:89–102

Kadowaki K, Ishige T, Suzuki S (1986) Differences in the characteristics of mitochondrial DNA between normal and male sterile cytoplasms of *Japonica* rice. Jpn J Breed 36:333–339

Kemble RJ (1987) A rapid, single leaf, nucleic acid assay for determining the cytoplasmic organelle complement of rapeseed and related *Brassica* species. Theor Appl Genet 73: 364–370

Kemble RJ, Bedbrook JR (1980) Low molecular weight circular and linear DNA in mitochondria from normal and male sterile *Zea mays* cytoplasm. Nature 284:565–566

Laughnan JR and Gabay-Laughnan S (1983) Cytoplasmic male sterility in maize. Annu Rev Genet 17:27–48

Lin SC, Yuan LP (1980) Hybrid rice breeding in China. In: Innovative Approaches to Rice Breeding. International Rice Research Institute, Manila, pp 35–51

Mignouna H, Virmani SS, Briquet M (1987) Mitochondrial DNA modifications associated with cytoplasmic male sterility in rice. Theor Appl Genet 74:666–669

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco pith cultures. Physiol Plant 15:473–497

Powling A (1981) Species of small DNA molecules found in mitochondria from sugarbeet with normal and male sterile cytoplasms. Mol Gen Genet 183:82–84

Rottmann WH, Brears T, Hodge TP, Lonsdale DM (1987) A mitochondrial gene is lost via homologous recombination during reversion of CMS-T maize to fertility. EMBO J 6:1541–1546

Shikanai T, Yang Z-Cr, Yamada Y (1987) Properties of the circular plasmid-like DNA B_1 from mitochondria of cytoplasmic male-sterile rice. Plant Cell Physiol 28:1243–1251

Virmani SS, Govinda Raj K, Casal C, Dalmacio D, Aurin PA (1986) Current knowledge of and outlook on cytoplasmic-genetic male sterility and fertility restoration in rice. In: Rice Genetics, Proc Rice Genet Symp. International Rice Research Institute, Manila, pp 633–647

Yamaguchi H and Kakiuchi H (1983) Electrophoretic analysis of mitochondrial DNA from normal and male sterile cytoplasm in rice. Jpn J Genet 58:607–611

Young J, Virmani SS, Khush GS (1983) Cyto-genic relationship among cytoplasmic-genetic male sterile, maintainer and restorer lines of rice. Philipp J Crop Sci 8:119–124