Theor Appl Genet (1988) 75:234–236

THEORETICAL AND APPLIED GENETICS

Mitochondrial DNA polymorphism in male-sterile cytoplasm of rice

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Received August 10, 1987; Accepted September 3, 1987
Communicated by K. Tsunewaki

Summary. Mitochondrial DNAs (mtDNAs) were isolated and purified from ten strains of rice plants with male-sterile cytoplasm. The mtDNAs were digested with the restriction endonuclease *Pst*I and the fragment patterns produced were analysed by 0.7% agarose gel electrophoresis. Restriction fragment length polymorphism was observed among the mtDNAs analysed; there were seven different patterns for the ten examined. Our results indicate that there are a variety of mtDNAs in cytoplasmically male-sterile rice.

Key words: Male-sterile cytoplasm – Mitochondrial DNA – Restriction fragment length polymorphism – Rice

Introduction

Cytoplasmic male sterility (CMS) is a maternally inherited trait common in higher plants that causes a plant to fail to produce functional pollen grains. To produce hybrid seeds in rice, CMS is a very useful character. It prevents the occurrence of self-fertilization and circumvents the costly procedure of hand emasculation. The male-sterile phenotype is caused by interactions between nuclear and cytoplasmic factors (Clayton 1950). Extensive surveys of mitochondrial DNA (mtDNA) variation have been done for many kinds of plants to examine the extent of polymorphism and relatedness of cytoplasms (Pring and Lonsdale 1985). Differences in mitochondrial nucleic acids are correlated with variation in the restoration of fertility in cytoplasms of the cms-S subgroup of maize (Sisco et al. 1985). In rice, however, there are only two reports on differences in the characteristics of mtDNA in normal and CMS rice (Yamaguchi and Kakiuchi 1983; Kadowaki et al. 1986). Comparison of rice mtDNAs in restriction fragments analysis has not been done. Male-sterile cytoplasms of rice were found in about 75 strains of *Oryza sativa, O. rufipogon,* and *O. glaberrima,* but in no strains of *O. barthii* or *O. breviligulata* (Shinjyo 1984). In 1970, the pathotoxin produced by *Helminthosporium maydis* race *T* seriously damaged hybrid corns (which contain *T* cytoplasm) and forced the industry to abandon its use. Numerous investigations have suggested that an inseparable association between disease susceptibility and male sterility appears to exist in the *T* cytoplasm (Umbeck and Gengenbach 1983). In order to minimize such damage, it is necessary to understand the characteristics of male-sterile cytoplasms of rice and to classify them into distinct groups differing in biochemical or biological aspect. In this study we used ten strains with male-sterile cytoplasm which are important in the production of hybrid seeds in rice, and which are currently being characterized genetically. The purpose of the work reported in this paper is to compare the restriction fragment patterns so as to examine the characteristics of the mtDNAs and to identify relationships among the strains.

Materials and methods

Definition of terms

CMS: cytoplasmic male sterility or cytoplasmically male sterile.

Plant materials

Ten strains of rice with male-sterile cytoplasm of different origins were used (Table 1).

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Table 1. Male-sterile cytoplasms of rice used as sources of mtDNA

Name of male-sterile cytoplasm	Species of cytoplasm donor	Type of male sterility	Source[a]	Reference
(cms-Bo)	*Oryza sativa*	gametophytic	R	Shinjyo 1975
(cms-R)	*Oryza rufipogon*	gametophytic	N	Maruyama (unpubl.)
(cms-UR89)	*Oryza rufipogon*	gametophytic	R	Shinjyo (unpubl.)
(cms-UR93)	*Oryza rufipogon*	gametophytic	R	Shinjyo (unpubl.)
(cms-UR102)	*Oryza rufipogon*	gametophytic	R	Shinjyo (unpubl.)
(cms-UR104)	*Oryza rufipogon*	gametophytic	R	Shinjyo (unpubl.)
(cms-UR106)	*Oryza rufipogon*	sporophytic	R	Shinjyo (unpubl.)
(WA)	*Oryza rufipogon*	sporophytic	N^I	Lin and Yuan 1980
(cms-Gam)	*Oryza sativa*	sporophytic	N^I	Lin and Yuan 1980
(MS-577)	*Oryza nivara*		N^I	Virmani et al. 1981

[a] R: University of the Ryukyus, Japan; N: National Agriculture Research Center, Japan; N^I: Supplied by S. S. Virmani, International Rice research Institute, Philippines

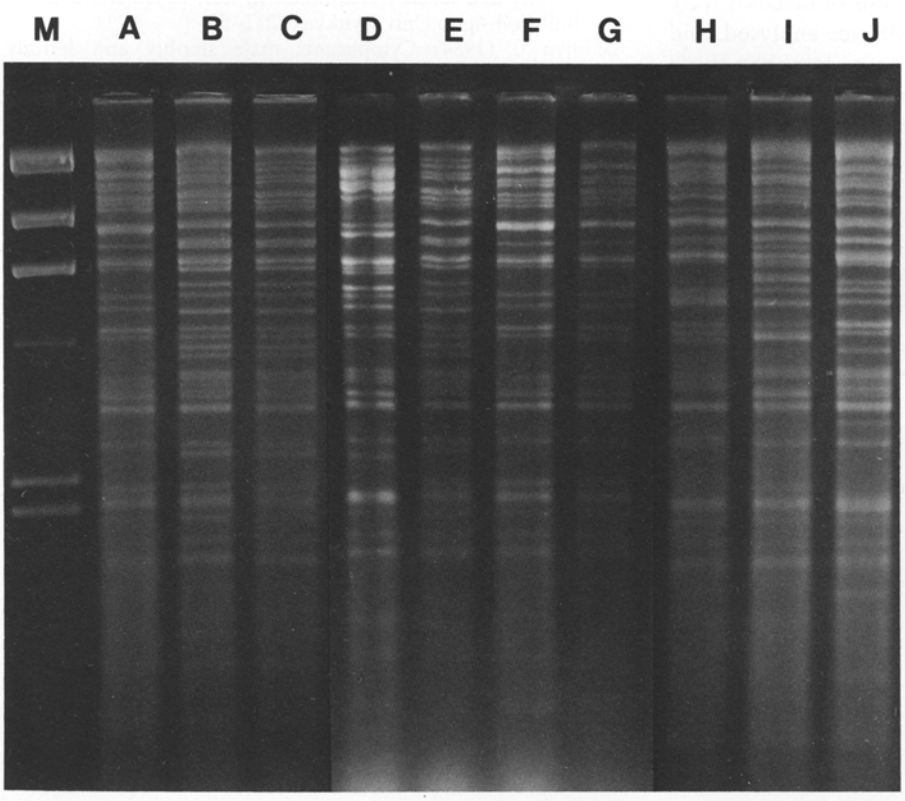

Fig. 1. Electrophoretic patterns of *Pst*I-digested mitochondrial DNAs from cytoplasmically male-sterile rice on 0.7% agarose gel. *Lanes: M* *Hin*dIII-digested lambda DNA; *A* (cms-Bo); *B* (cms-UR89); *C* (cms-UR102); *D* (cms-R); *E* (cms-UR93); *F* (cms-UR104); *G* (cms-UR106); *H* (WA); *I* (cms-Gam); *J* (MS-577)

MtDNA analysis

Rice seeds were germinated in the dark on two layers of cheesecloth in a container at 28 °C for two weeks. MtDNA isolation and purification, restriction endonuclease analysis, and agarose gel electrophoresis were done as described previously (Kadowaki et al. 1986).

Results and discussion

The mtDNAs of ten strains of CMS rice were prepared and examined by restriction endonuclease analysis. As shown in Fig. 1, the patterns of *Pst*I- digested fragments were the same for cms-Bo and cms-UR104, and also for cms-UR89, cms-UR93, and cms-UR102 cytoplasms. Other cytoplasms had fragment patterns that did not resemble any others. Thus, there were seven different patterns for the ten examined. We did not detect any difference in the fragment pattern by *Bam*HI, *Eco*RI or *Xba*I analysis for cms-Bo and cms-UR104, or for cms-UR89, cms-UR93, and cms-UR102 cytoplasms (data not shown). It is too early to draw conclusions about the evolutionary relationships among the ten strains of CMS rice from these results alone. However, we speculate that the smaller the mtDNA diversity, the closer the phylogenetic relationship. It is noteworthy that several cytoplasms derived from different origins

had the same restriction profile for mtDNA. These cytoplasms will provide useful information for the study of the evolution of CMS.

Summation of the sizes of the rice mtDNA restriction fragments gave genome sizes of about 174 kilobases of a unique sequence, and 280 kilobases when multiple bands were included in the calculation. The number of fragments of rice mtDNA produced by *Pst*I or *Bam*HI digestion (data not shown) was much smaller than that of maize mtDNA (Borck and Walbot 1982). These results suggested that the molecular weight of rice mtDNA is smaller than that of maize mtDNA, and that the rice mtDNA is less complex (Lonsdale et al. 1984).

In conclusion, seven different kinds of mtDNA were found among the ten strains of CMS rice analysed, and their size and complexity seemed to be less than those of maize mtDNA. In the near future, most of male-sterile cytoplasms of rice will be classified into distinct groups by the restorer genes of fertility and by this restriction endonuclease analysis.

Acknowledgements. The authors thank Dr. S. S. Virmani (IRRI, Philippines) and Dr. K. Maruyama (NARC, Japan) for useful discussion. This work was supported in part by a grant, "Rice Breeding Project (No. 12122)", from the Ministry of Agriculture, Forestry and Fisheries (1986).

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