Differences in the Characteristics of Mitochondrial DNA between Normal and Male Sterile Cytoplasms of Japonica Rice

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We attempted to analyse the mechanism of cytoplasmic male sterility (cms) in two strains of rice, that is, cms-Bo Taichung 65 with cytoplasm of cultivar Chinsurah Boro II and cms-R Reimei with cytoplasm of a strain of Oryza rufipogon. Mitochondrial DNAs (mtDNAs) were purified from cms and fertile (normal) rice varieties and the electrophoretic pattern of their restriction fragments was compared. MtDNA purified from cultivar Chinsurah Boro II cytoplasm contained two plasmid-like low molecular weight DNAs and the pattern of the restriction fragments of mtDNA from Chinsurah Boro II cytoplasm was clearly different from that of the normal cytoplasm. Plasmid-like DNAs have not been detected yet in the mtDNA from a strain of Oryza rufipogon cytoplasm but the pattern of the restriction fragments of mtDNA was also obviously different from that of the normal cytoplasm. Moreover the pattern of the restriction fragments of mtDNAs from a strain of Oryza rufipogon and Chinsurah Boro II cytoplasm was clearly different. In contrast, the pattern of the restriction fragments of chloroplast DNAs from normal and cms cytoplasms of Taichung 65 or Reimei was identical. We speculate that the difference in the mitochondrial genome between cms and normal cytoplasms of Taichung 65 or Reimei is, at least partly, responsible for the occurrence of cms.

KEY WORDS: Oryza sativa L., Oryza rufipogon Gsw., rice, mitochondrial DNA, cytoplasmic male sterility, plasmid-like DNAs.

Introduction

Cytoplasmic male sterility (cms) which interferes with pollen maturation is a maternally inherited trait and widely distributed among many higher plants. To produce a F₁ hybrid plant, cms is a very useful character as it enables to prevent the occurrence of self-fertilization. Several types of cms have been identified in rice by Katsuo and Mizushima (1958), Kitamura (1958) and Shinjo (1972) and sources of cytoplasm in rice have been classified into four groups on the basis of certain nuclear genes called "restorers of fertility" that suppress the expression of male sterility (C. Shinjo, data unpublished). Molecular analysis of cms has been actively pursued in many plants and differences in the characteristics of mitochondrial DNA (mtDNA) between cms and fertile lines of a number of species have been described (Belliard et al. 1978, Pring and Levings 1978, Thompson et al. 1980, Galun et al. 1982, McNay et al. 1983, Palmer et al. 1983, Pring and Lonsdale 1985). However the cause of cms has not been documented yet. Only one report dealing with the molecular analysis of cms in rice (Yamaguchi and Kakuuchi 1983) demonstrated that mtDNA which was isolated from the callus derived from cms-Bo Taichung 65 possessed two low molecular weight DNA species.

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In this paper we used two rice strains with cms, i.e. Taichung 65 and Reimei. Our purpose was to study the organelle DNA in applying the restriction endonuclease fragment analysis to clarify the mechanism of cms in rice.

Materials and Methods

Plant materials
Cms-Bo Taichung 65 with male sterile cytoplasm of cultivar Chinsurah Boro II was originally developed by C. Shinjiyo (1969). Restorer gene was characterized by C. Shinjiyo (1969, 1975). Taichung 65, Reimei and cms-R Reimei with male sterile cytoplasm of a strain of Oryza rufipogon were kindly supplied by the Heterosis Breeding Laboratory (NARC, Tsukuba, Japan). Restorer gene for the cms-R cytoplasm was found in some indica cultivars (K. Maruyama personal communication).

Isolation of mitochondrial DNA
Thirty grams of rice leaves (ca. 10 cm long) were harvested and minced with a knife and briefly blended in the 150 ml of homogenization buffer (0.4 M sucrose, 50 mM Tris-HCl pH 7.5, 5 mM Na2EDTA, 0.1% bovine serum albumin, 5 mM 2-mercapto-ethanol). The homogenate was filtered through four layers of gauze and two layers of miracloth and centrifuged for 10 min at 1,000 × g. The supernatant was collected and centrifuged for 10 min at 17,000 × g. The following method employed to isolate mtDNA was the same as that applied by Umbeck and Gengenback (1983).

Restriction enzyme reaction and agarose gel electrophoresis
Restriction enzymes were purchased from TAKARA Shuzo Co., Ltd. Enzyme reaction was carried out according to the instruction manual except that the reaction time was 3~4 h. Ten μl of DNA solution as previously prepared was used in each reaction and the reaction mixture contained 10 μg of RNase. Then the DNA samples were separated by electrophoresis on 1% or 0.7% agarose gel (Sigma, type II) with the running buffer containing 0.089 M Tris, 0.089 M boric acid, 0.002 M Na2EDTA, (pH 8.3) at 1.5 volts per cm for 15 h in a submarine type electrophoresis apparatus. The gel was stained for 15 min in 100 μg ethidium bromide per 100 ml of deionized water and was rinsed for 10 min in deionized water and photographed with a Polaroid Type 665 or 667 film under a UV illumination lamp.

Film scanning
Polaroid negative film was scanned by using a densitometer (Toyo, DMU-33 C) with a slit 0.2 mm wide.

Isolation of chloroplast DNA
Chloroplast DNA (cpDNA) was isolated by the same method as that applied by Hirai et al. (1985).

Results and Discussion
We purified and analysed mtDNAs from normal and cms-Bo Taichung 65. As reported by Yamaguchi and Kakiuchi (1983) mtDNA from cms-Bo Taichung 65 had two plasmid-like low molecular weight DNAs which were absent in normal Taichung 65, and the electrophoretic pattern of the fragments digested with XhoI or PstI was different from that of the normal type cytoplasm (Fig.1, 4). Nawa et al. (1985) reported
that the two plasmid-like DNAs consisted of covalently closed circular molecules unlike the S1 or S2 linear plasmid DNA found in cms-S maize. Some strains of Latin American maize which have plasmid-like DNAs such as R-1, R-2 are male-fertile (WEISSINGER et al. 1982) and several authors have reported that plant mitochondria harbour low molecular weight DNAs (Powlíng 1981, HANDA et al. 1984). Furthermore plasmid-like DNA was found in other species i.e. mushroom (MOHAN et al. 1984) and yeast (KOVAC et al. 1984). Therefore the assumption that plasmid-like DNAs in rice are associated with cms seems to be not necessarily true, though such a presence of plasmid-like DNAs is an interesting phenomenon which may enable to distinguish between various cytoplasts. In the case of cms-R and normal Reimei, mtDNA digested with XhoI or PstI showed also differences in the pattern of fragments. Plasmid-like low molecular weight DNA had not been detected yet in mtDNA from cms-R Reimei (Fig. 2). BOESMORE et al. (1985) characterized the mtDNA fragment in which the mitochondrial genomes from the cms line and fertile line differed from each other. They showed that the particular DNA arrangement occurred
Fig. 2. Agarose gel electrophoretic pattern (left) and diagram (right) of rice mtDNAs.  
Lanes A, D, G, phage lambda DNA digested with HindIII as size marker;  
Lane B, mtDNA from normal Reimei;  
Lane C, mtDNA from cms-R Reimei;  
Lane E, mtDNA from normal Reimei digested with Pst I;  
Lane F, mtDNA from cms-R Reimei digested with Pst I;  
Lane H, mtDNA from normal Reimei digested with Xho I;  
Lane I, mtDNA from cms-R Reimei digested with Xho I.  
Open arrowheads indicate additional band compared with normal cytoplasm.  
Solid arrowheads indicate missing band compared with cms-R cytoplasm.

Fig. 3. Agarose gel electrophoretic pattern (left) and diagram (right) of rice mtDNAs.  
Lane A, phage lambda DNA digested with HindIII as size marker;  
Lane B, mtDNA from cms-Bo Taichung 65 digested with Bam HI;  
Lane C, mtDNA from cms-R Reimei digested with Bam HI.

Fig. 4. Agarose gel profile of mtDNA fragments from normal and cms-Bo Taichung 65 digested with Pst I.  
Lane A, phage lambda DNA digested with HindIII as size marker;  
Lane B, mtDNA from cms-Bo Taichung 65 digested with Pst I;  
Lane C, mtDNA from normal Taichung 65 digested with Pst I.  
Negative film obtained in this experiment was scanned with a densitometer.  
Panels D and E represent a profile of the pattern of the DNA restriction fragments from lanes B, C respectively.  
The top of the gels is on the left hand side and the bottom is on the right hand side.  
One peak of this figure represents one DNA fragment. The concentration of the DNA fragment reflects the area of peak.
in the specific fragment which segregated with the cms phenotype. We assume that the site specific recombination which is different from fertile and consequently divergent from that of the fertile line may have occurred in the mtDNA of cms rice. Thereafter we compared the mtDNAs from cms-R Reimei and cms-Bo Taichung 65. We observed that the pattern of the mtDNA fragments digested with *Bam*HI was obviously different in cms-R Reimei and cms-Bo Taichung 65 (Fig. 3). This observation suggests that the mitochondrial molecules are different in cms-R Reimei and cms-Bo Taichung 65.

Based on film scanning investigations on the pattern of the restriction fragments in cms-Bo and normal Taichung 65 (Fig. 4), it was shown that the pattern of the DNA fragments and the copy number of the fragments were significantly different. This phenomenon was presumably related to the presence of repeated sequences and heterogeneous mtDNA molecules in rice. Such a pattern was similar to that reported in maize (Lonsdale et al. 1984).

CpDNAs from cms-R and normal Reimei, which were isolated and digested with *Pst* I (Fig. 5) or *Xho*I (data not shown), were analysed electrophoretically. No distinguishable differences in the pattern of the DNA fragments was detected between the two types of cytoplasms. In the case of the cpDNA from cms-Bo and normal Taichung 65, there were no differences in the pattern of the restriction fragments digested with *Pst* I or *Xho*I (data not shown).

From the above results it is concluded that the molecules of the chloroplast genome could not be distinguished while those of the mitochondrial genome were different between normal and cms cytoplasms when DNA analysis by endonuclease digestion was applied. It is thus assumed that the occurrence of cms may be ascribed at least partly to the differences in the molecules composing the mitochondrial genome which were revealed in cms-R Reimei and cms-Bo Taichung 65. The application of these techniques may be useful to identify differences in cytoplasms. Studies on the mechanism of occurrence of cms using *in vitro* analysis of mitochondrial protein synthesis are currently under way.

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日本稲における雄性不稔細胞質及び正常細胞質
由来のミトコンドリア DNA の差異

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細胞質雄性不稔（cms）の機構の研究は、トウモロコシを中心に数多くの植物で精力的に行なわれており、トウモロコシでは核による雄性回復因子により T，C，S の 3 つの雄性不稔細胞質と正常細胞質 N に大別されている。また制限酵素を使った DNA 切断パターン解析、ミトコンドリアの試験管内蛋白質合成解析など分子レベルでの研究も進められており、これらの結果からミトコンドリアに雄性不稔の因子があると考えられている。現在までイネでは数種類の細胞質で雄性不稔現象が同定されており、核による雄性回復因子によりいくつかのグループに分けられつつある。ところが分子レベルでの研究は、他の植物と比較した場合立つ遅れており、本研究では Chinsurah Boro II 由来の細胞質をもつ cms-Bo 台中 65 号と Oryza rufipogon 由来の細胞質をもつ cms-R レイメイを材料にして、制限酵素を用いた DNA 切断パターン解析により、cms の原因を探った。幼葉よりミトコンドリア DNA（mtDNA）、葉緑体 DNA（cpDNA）を単離精製し、数種類の制限酵素を用い DNA の切断パターンを比較した。cpDNA では雄性不稔細胞質と正常細胞質の間で DNA 切断パターンに差が見出せるのに対し、mtDNA では両者の間に明らかな差が見出された。また制限酵素処理しない cms-Bo 台中 65 号の mtDNA では、幼葉においても、分子量の小さい 2 種類の DNA 分子が観察された。それに対し現在までのところ cms-R レイメイの細胞質では、そのような小分子は発見出来なかった。また cms-Bo 台中 65 号と cms-R レイメイの mtDNA は切断パターンが異なっていた。これらのことから、我々イネの cms の原因の少なくとも 1 つはミトコンドリアにあると推察されること、そして cms-Bo 台中 65 号と cms-R レイメイは DNA レベルで異なるミトコンドリア分子をそれぞれの細胞質にもつことを明らかにした。