SHORT PAPER

Electrophoretic analysis of mitochondrial DNA from normal and male sterile cytoplasms in rice

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ABSTRACT

Mitochondrial DNA was isolated from rice with normal (fertile) and male sterile cytoplasms were analyzed by electrophoresis, the patterns were readily distinguishable between the two cytoplasmic types. It was found that mitochondria from male sterile cytoplasm contain DNA species of sizes 1.5 kilobase pairs (kb) and 1.2 kb, in addition to the DNA of relatively much higher molecular weight. In contrast mitochondria from normal cytoplasm contained only one of these DNA species, that of higher molecular weight.

1. INTRODUCTION

Cytoplasmic male sterility (cms) has been extremely important in the widespread production of hybrid seed. In rice, cytoplasmic male sterility was reported by Shinjo and Omura (1966). In B_1F_1 population of (Chinsurah Boro II $\times$ Taichung 65) $\times$ Taichung 65, partial and complete male sterilities segregated in a ratio of 1:1. Furthermore, Shinjo (1969) produced the BT^u line by back-crossing repeatedly to the partially male sterile F_1 hybrids of Chinsurah Boro II $\times$ Taichung 65 with Taichung 65 as the recurrent parent, and showed that the BT strain has sterile cytoplasm while Taichung 65 normal cytoplasm. When a nuclear restorer gene was introduced into the sterile cytoplasm, the F_1 plants had a 90% or more seed set. Such cms source has been widely used for the seed production of hybrid rice (Yang *et al.* 1982). However, no molecular studies of cytoplasmic male sterility in rice have been reported.

In maize, on the other hand, cms sources have been classified into four groups, N or normal and T, C, and S-cms, based on their response to nuclear restorer genes (Duvick 1965). Factors responsible for cms of maize are located in the mitochondrial DNA (mtDNA). Levings and Pring (1976) found that the DNAs are different between the N and T cytoplasms by restriction endonu-

1) Boro cytoplasm with Taichung 65-nucleus.

lease fragment analyses of their mtDNAs. Moreover, Pring *et al.* (1977) detected two small mtDNAs by electrophoresis in the S cytoplasm of male sterile maize. When some cms-S strains reversed spontaneously to male fertility, either of the small mtDNA, S-1 or S-2, disappeared from their cytoplasm. DNA-DNA hybridization showed that S-2 is capable of transposition to a mitochondrial chromosomal DNA and involved in the mtDNA rearrangements (Levings *et al.* 1980).

Recently, small DNAs associated with cms trait were described in mtDNA from sorghum (Pring *et al.* 1982). We report here that small DNAs which may be involved in the cms trait were detected in rice mtDNA.

2. MATERIALS AND METHODS

Strains

Seeds of the BT line and Taichung 65 were kindly supplied by Prof. C. Shinjo, University of the Ryukyus. BT line has the cytoplasm of Chinsurah Boro II and the same genetic background as that of Taichung 65.

Callus Formation

Because of a few seeds of the BT line, rice cells were propagated by induction of callus formation. The seeds were gotten rid of the glumes, sterilized with 5% sodium hypochlorite for 1 hr and rinsed 5 times with sterile water. Each seed was inoculated on the 10 ml Miller medium supplemented with 0.01 mM 2, 4-D in a 30 ml test tube. Calluses of 5 to 7 weeks after induction were transplanted on 50 ml fresh medium in a 100 ml flask. Repeatedly after 5 to 7 weeks in culture the calluses were subcultured on medium for their propagation. The cultures were kept in complete darkness at 30°C.

Isolation of mtDNA

Isolation techniques used in this experiment were modifications of procedures described by Kolodner and Tewari (1972). Calluses were homogenized in 10 ml/g fresh weight 0.3 M mannitol, 0.05 M Tris-HCl, pH 8.0, 3 mM Na₂ EDTA, 1 mM mercaptoethanol and 0.1% bovine serum albumin using a mortar. Homogenates were filtered through two layers of nylon cloth. The filtrate was centrifuged twice at $1,520 \times g$ for 10 min. The resulting supernatant was recentrifuged at $7,000 \times g$ for 5 min. Mitochondria were monitored by their Brownian movement observed under microscope. The mitochondrial pellet was resuspended in 10 ml of homogenization buffer. DNase was added to the suspension to give final concentration of 50 $\mu\text{g/ml}$, followed by incubation at 4°C for 1 hr.

Mitochondria were added to the washing medium containing 0.3 M sucrose,

Fig. 1. Agarose gel electrophoretic patterns of mitochondrial DNA of normal cytoplasm (A, C), mitochondrial DNA of male sterile cytoplasm (B, D), bacteriophage lambda DNA digested with *Eco*RI (E), and undigested lambda DNA (F).

50 mM Tris-HCl, pH 8.0, and 20 mM Na₂EDTA, and washed twice by centrifugation at 12,000×g for 10 min. The final pellet was lysed in 50 mM Tris-HCl, pH 8.0, 20 mM Na₂EDTA, 10% sodium sarkosyl and 500 µg/ml pronase E at room temperature for 10 min. The DNA was purified by three phenol-chloroform extractions and ethanol precipitation. Precipitated DNA was resuspended in 100 µl of 10 mM Tris-HCl, pH 8.0, 10 mM KCl and 0.1 mM Na₂-EDTA.

Agarose Electrophoresis

The mitochondrial DNA was electrophoresed at room temperature in 0.7 or 1.0% agarose (Sigma, type II) gels for 15 hr at 2.0 V/cm. Electrophoresis buffer was 40 mM Tris, pH 7.9, 5 mM sodium acetate, 1 mM Na₂EDTA containing 0.2% sodium dodecyl sulfate and 0.02% bromophenol blue. After electrophoresis, the gels were soaked in 0.5 µg/ml ethidium bromide for 30 min. Gels were photographed using a UV filter.

Molecular-Weight Determination

Bacteriophage lambda digested with *Eco*RI restriction endonuclease (Takara Shuzo Co., Ltd.) was used for molecular-weight marker (Daniels *et al.* 1980).

3. RESULTS

Isolated mitochondrial DNAs of normal and male sterile cytoplasms were electrophoresed in agarose gels with *Eco*RI-digested lambda DNA as molecular-weight markers. Agarose gel electrophoresis of rice mtDNA showed a main broad band of high molecular-weight DNA from both cytoplasms examined (Fig. 1). Main mtDNAs from either normal or male sterile cytoplasm had the same distance migrated as lambda DNA.

Male sterile cytoplasm examined yielded two additional bands (Fig. 1B, D). But, normal cytoplasm did not yield these unique DNAs associated with sterile cytoplasm (Fig. 1A, C). When logarithm of molecular weight was plotted against distance migrated, the two unique DNAs from male sterile cytoplasm were estimated to have molecular weights of ca. 1.0×10^6 (1,500 bp) and 0.8×10^6 (1,200 bp), respectively. The male sterile cytoplasm originated in Chinsurah Boro II, a variety from the Boro rice group, hence we have designated these unique DNAs as the B-1 and B-2 DNAs of rice.

4. DISCUSSION

The investigation reported here shows that there is a difference between the mtDNAs isolated from the calluses of male sterile and fertile cytoplasms in rice. Two small mtDNA species are present in the male sterile cytoplasm. Therefore, rice is similar to maize (Pring *et al.* 1977) and sorghum (Pring *et al.* 1982) in that mtDNA species are different between normal and male sterile cytoplasms.

Although we cannot disregard chloroplasts or other cytoplasmic DNA's as potential carriers of cytoplasmic male sterility, the electrophoretic data supply an evidence that the mitochondria are the organelle involved in the cytoplasmic inheritance. If the presence of the two small mtDNA species does cause cms, it may be possible to convert artificially normal rice cytoplasm to male sterility. We anticipate that the results described here will be of considerable help in recognizing and exploiting cytoplasmic variation in rice.

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