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Comparative Study on Mitochondrial DNA of T-type Cytoplasmic Male Sterile Lines and Their Maintainers in Wheat (*Triticum aestivum* L.)

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Abstract mtDNAs of T type wheat cytoplasmic male sterile lines Ning Drawf 14 (ND14) and their maintainers Ning Drawf 13 (ND13) were isolated and digested completely with restriction endonucleases EcoRI, PstI, EcoRV, BamHI. The results revealed that the molecular structure of mtDNAs from ND14 and ND13 cytoplasms were significantly deviated. The mitochondrial genomic difference between CMS line and maintainers were uncovered by southern hybridization with probes of 18S+5S rRNA、atpA genes from wheat and pea mitochondria, respectively. Due to the complexity of mtDNA and no proof of protein difference, it has not yet been demonstrated whether mtDNA difference of Normal and Male Sterile Cytoplasm of wheat is associated with CMS.

Key words *Triticum aestivum* L.; T type Cytoplasmic Male Sterility; Mitochondrial DNA; Southern hybridization

Since 1970s, relation of plant mitochondrial genome with cytoplasmic male sterility (CMS) had been investigated, and few studies on CMS principle of wheat were conducted. By molecular hybridizing of atpA gene with mtDNA digested by restriction endonuclease, the relationship of atpA of rice and sunflower with CMS had been revealed (Hanso, M. R, 1991; Lusia, S. et al., 1988), However, few discussions about the effects of mitochondrial 18S+5S rRNA gene, atpA gene on CMS were reported in wheat.

Restriction patterns of mtDNA of cytoplasmic male sterile line and maintain line in wheat were analyzed and compared in the paper, the relationship of mitochondrial atpA gene, 18S+5S rRNA gene with CMS principle of wheat was discussed primarily.

1 MATERIALS AND METHODS

1.1 Materials

Wheat seeds (male sterile ND14 and maintain line ND13) were obtained from Professor Zhao Yinhuai, Institute of Cereal Plant, Jiangsu Academy of Agricultural Science.

Probes, which are wheat mitochondrial (mt) 18S+5S rRNA gene (1.5 kb) and pea mitochondrial atpA gene (3.2 kb), were provided by Professor K. Tsunewaky, Kyoto University, Japan.

1.2 Methods

1.2.1 Isolation and Purification of mtDNA

mtDNA was prepared according to Phillip, E (1986).

1.2.2 Restriction endonuclease digestion and electrophoresis

mtDNA (3–5 μg) were digested for 6 hours with EcoRI, PstI, EcoR V, BamHI respectively.

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The enzyme concentration used was 3–4 units / μg mtDNA. Restriction fragments were separated by electrophesis on 0.8% agarose gels for 15 hours. Electrophoretic buffer was TAE (40 mM Tris, 20 mM Aceticacid, 1 mM EDTA). After electrophesis, DNA fragments were stained with ethidium bromide for 15 minutes and photographed under ultraviolet light.

1.2.3 Southern hybridization

^{32}P–labeled probe DNA was prepared by nick–translation (Maniatis, T. E. et al., 1982). Southern hybridization had been described (Southern, 1975).

2 RESULTS

2.1 Comparison of mtDNA restrication fragments patterns between ND14 and ND13

After mtDNA was digested with restrication endonuclease and electrophesised, the results indicated that had difference of restrication patterns between ND14 and ND13. Fig. 1 was the patterns of mtDNA restricted with EcoRI and PstI. Fig. 2 was the patterns of mtDNA restricted with EcoR V, BamHI.

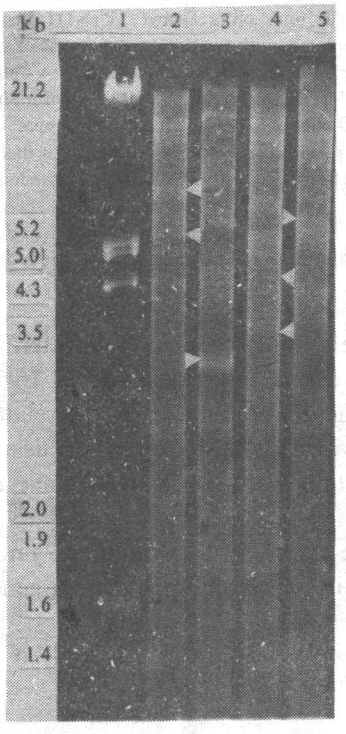

Fig. 1 EcoRI and PstI restriction fragment patterns of mtDNAs (Arrows indicate the diversities.)
1. DNA Molecular weight Marker 2, 4. Male Sterile line 3, 5. Maintainer line 2, 3. Digested by EcoRI 4, 5. Digested by Pst I

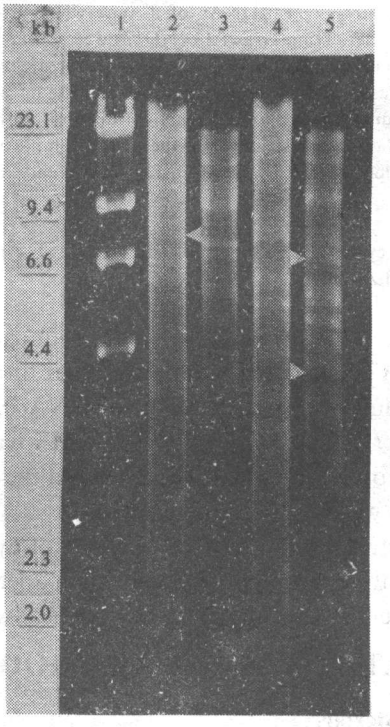

Fig. 2 EcoRV and BamHI restriction fragment patterns of mtDNAs(Arrows indicate the diversities.)
1. DNA Molecular weight Marker 2, 4. Male Sterile line 3, 5. Maintainer line 2, 3. Digested by EcoRV 4, 5. Digested by BamH I

2.2 atpA diversity of mitochondrial genome between ND14 and ND13

Hybridization with the pea mitochondrial atpA probe showed that atpA copy number was different in mitochondrial genome between ND14 and ND13 (Fig. 3, Fig. 4). The results were divided into two kinds: one was three hybridized bands of sterile line and two of maintain line, such

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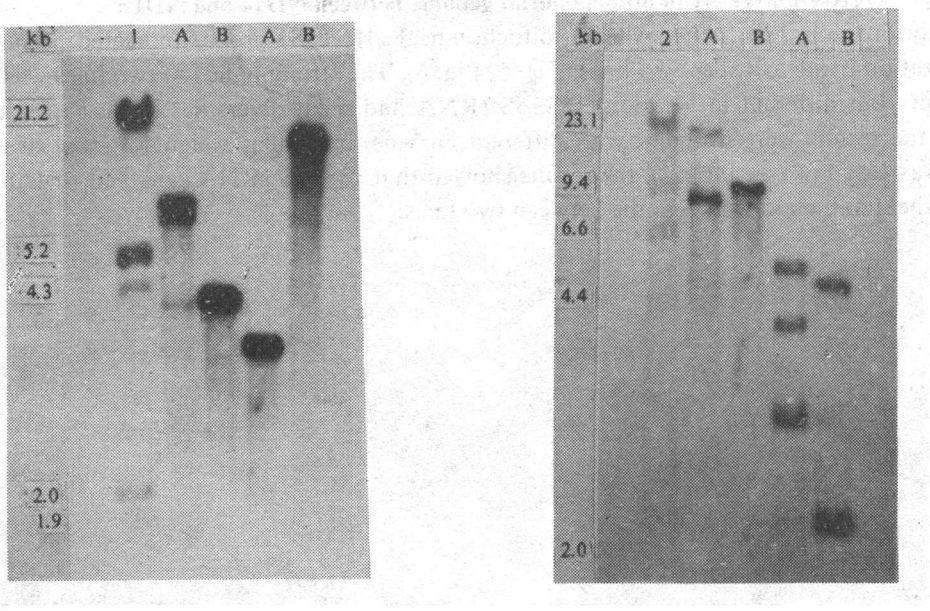

Fig. 3, 4 Hybridization of probe atpA to digested mtDNAs.
Fig. 3 Digested by EcoR I (Left) and Pst I (Right).
Fig. 4 Digested by EcoRV (Left) and BamH I (Right).
1, 2. Hybridization of λDNA to DNA Molecular Weight Marker III and Marker II.
A. Male Sterile line; B. Maintainer line.

as restricted with EcoRI and PstI; the other was two hybridized bands of sterile line and one of maintain line, such as restricted with EcoRV and BamHI. Size of hybridization fragments was showed in table 1. The results uncovered that atpA had diversity in mitochondrial genome between two lines.

Table 1 Size of hybridization fragments (kb) of atpA, 18S+5S rRNA gene to mtDNA digested by EcoRI, Pst I, BamHI and EcoRV.

	EcoRI		PstI		EcoRV		BamHI	
Gene	A	B	A	B	A	B	A	B
atpA	7.37	7.37	11.0	11.0	17.13	9.3	5.32	4.9
	6.54	3.9	3.25		8.68		4.0	2.4
	3.9						2.88	
18S+5S rRNA	13.0	10.64	15.65	19.5	18.5	13.5	3.5	5.23
	7.0	7.0	7.7	11.16	13.5	6.13	3.1	3.3
			5.6	5.9				
	4.56	3.1	3.5		7.6		2.8	
	3.3		3.15		6.9		2.63	
			2.48		5.2			
			2.2	4.9				
				4.2				
				4.0				
				3.4				
				3.2				

Note: added "—": feeble band of hybrization.
A. male sterile line; B. maintainer line

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2.3 18S+5S rRNA diversity of mitochondrial genome between ND14 and ND13

Hybridization with the wheat mitochondrial 18S+5S probe indicated diversities in hybridization bands between two lines (Fig. 5, Fig. 6). The strong bands of two lines was the same in number but different in location. 18S+5S rRNA had great diversities in the feeble bands, of course, the results were different with different enzyme, molecular weight of most of the feeble bands was very low (see table 1). the results showed that 18S+5S rRNA gene had structural diversity in wheat mitochondrial genome between two lines.

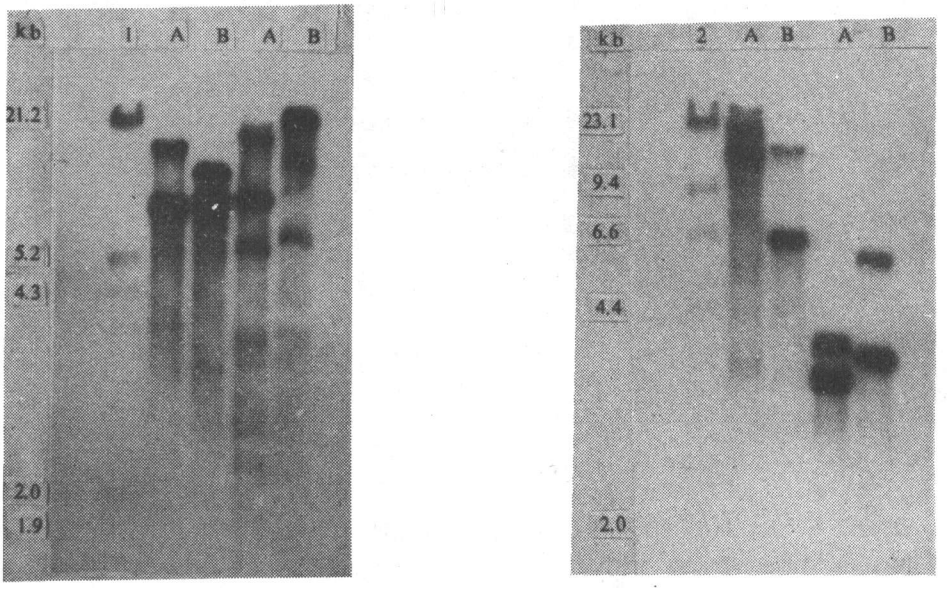

Fig. 5, 6 Hybridization of probe 18S+5S rRNA to digested mtDNAs
Fig. 5 Digested by EcoRI (Left)and PstI (Right).
Fig. 6 Digested by EcoRV (Left)and BamHI (Right).
1, 2. Hybridization of λ DNA to DNA Molecular. Weight Marker III and Marker II.
A. Male Sterile line; B. Maintainer line.

3 DISCUSSION

Recent studies indicated that cytoplasmic male sterility (CMS) was associated with mtDNA (Dewey, R. E., 1987;Hanson, J. C. et al., 1986; Pruitt, K. D. 1991). Differences of restriction patterns between sterile and maintain line are revealed by our experiments, when mtDNA is digested by EcoRI, PstI, BamHI and EcoRV respectively. It is shown that CMS of wheat is relevant to mtDNA.

Plant mtDNA contained repeat sequences, recombination between repeat sequences made mtDNA variation (Andre, C. et al., 1992; Fragoso, L, 1989; Mackenzie, S. A. et al., 1990; Quetier, F, et al., 1977). When function gene is located at or near repeat sequences, it may to rearrange and cause function gene alteration in structure. Most of CMS was caused by mtDNA recombination (Hanson, M. K, 1991). It was discovered that atpA gene, 18S+5S rRNA gene, which were located at or near repeat sequences, were multiple copies in mitochondrial genome, thus participating in recombination (Wang, 1990; Lusio, S. et al., 1988;Stern, D. B, 1984). We find that atpA, 18S+5S rRNA gene are multiple copies, with diversity of structure. atpA copy number of sterile line differs from that of maintain line. 18S+5S rRNA gene is also multiple copies, with two copies of 18S+5S rRNA gene in strong hybridization bands of sterile and maintain line. 18S+5S rRNA, gene have

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not only strong bands but also feeble bands. It is suggested that 18S+5S rRNA, atpA gene are located at or near repeat sequences, and rearrangement of repeat sequences leads to alteration of the 18S+5S rRNA, atpA gene in structure.

Previous studies indicated that diversities among polypeptide generated by mitochondrial translation in vitro of sterile and maintain gene may be determined by different fragments of mtDNA between two lines. For example, there was a different fragment in mtDNA of maize CMS-T, named BamHI fragment, a T-urf_{13} gene synthesizing 13 kD polypeptide was in BamHI fragment, whereas 13kD polypeptide was different between sterile and maintain line. It was discovered by sequence analysis that T-urf_{13} was formed by recombination, with 26S rRNA partial sequence (Levings, C. S. et al., 1989). This study concludes that there is 18S+5S rRNA gene altered partial sequences in the variation fragment of the sterile and maintain line of wheat. The change of 18S+5S rRNA gene could not only influence synthesis of the mitochondrial polypeptide, and disrupt structure and function of the mitochondria, but also make energy supply insufficient and pollen abortive that leads to CMS.

It is pointed out by the Research on wheat T-CMS that wheat CMS was associated with the abnormality of energy metabolism (Du, E. B, 1975). This study shows that atpA gene of the wheat mitochondria has been rearranged, the sterile line always has one more hybridized band compared with maintain line. It is suggested that atpA gene recombination can destroy mitochondrial energy metabolism and pollen development that leads to CMS.

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普通小麦（*Triticum aestivum* L.）T 型细胞质雄性不育系及其保持系的线粒体 DNA 比较研究*

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