Mitochondrial genome of rapeseed (*Brassica napus* L.).

1. Intraspecific variation of mitochondrial DNA

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(Received 23 October 1989)

ABSTRACT

Restriction endonuclease analysis was carried out for mitochondrial (mt) DNAs of 27 accessions of rapeseed (*Brassica napus* L.) to detect intraspecific variation of the mitochondrial genome. MtDNA isolated from green leaves was digested with ten endonucleases and was electrophoresed using agarose slab gel. Mt genomes of the 27 accessions were classified into two major types, each of which was further divided into three subtypes based on the length differences in the restriction fragments of mtDNAs. In total, six mt genome types (type Ia, Ib, Ic, IIa, IIb and IIc) could be identified. To analyze the relationship between the mt genome types and the cytoplasmic male sterility (cms) induction, restriction fragment patterns of two male sterile cytoplasms, namely, *nap* cms and *pol* cms, were compared with those of the normal cytoplasm. The mt genome of the *nap* cytoplasm was found to be either of Ia, Ib and Ic types, whereas the *pol* cytoplasm carried the type IIb mt genome. Mitochondrial linear plasmid DNA was also observed in several accessions, but the relationship between its presence and the cms trait could not be established.

1. INTRODUCTION

Cytoplasmic male sterility (cms) was detected in F₂ progenies of intraspecific crosses between rapeseed (*Brassica napus* L.) varieties by Shiga and Baba (1971, 1973) and Thompson (1972) (*nap* cytoplasm). Fu (1981) also observed that the cytoplasm of a Polish variety "Polima" induced male sterility (*pol* cytoplasm). Thus, the cytoplasms of rapeseed varieties had been classified into three groups, namely, *nap*, *pol* and normal cytoplasm.

Recently a molecular genetic approach to organellar genomes such as chloroplast (ct) and mitochondrial (mt) genome has been well developed. Therefore it has become possible to elucidate the phylogenetic relationships between genera, species or lines of higher plants based on the analysis of organellar genomes.

In the genus *Brassica*, the phylogenetic relationship has been considered based on the restriction endonuclease analysis of ctDNA (Erickson et al., 1983; Palmer et al., 1983a) and mtDNA (Palmer, 1988). Intraspecific variation of the ct

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genome in rapeseed was reported by Erickson et al. (1983), Palmer et al. (1983a) and Ohkawa and Uchimiya (1985). Also mt genome variation among some cms-inducing cytoplasms in rapeseed was described by Erickson et al. (1986).

Though the cytoplasmic differentiation on the cms trait among rapeseed varieties can be clearly detected, studies on the relationship between the cytoplasmic differentiation and the mt genome variation in rapeseed had not been carried out systematically using a large number of rapeseed varieties.

In this report, we performed a restriction endonuclease analysis of mtDNAs of 27 rapeseed accessions in order to clarify: (1) the extent of mtDNA variation within rapeseed, and (2) the relationship between mtDNA variation and the cms trait.

Table 1. Materials used as the source of mtDNA

Accession number	Cultivar	Origin
Euplasmic line (cultivar)		
1	Mali	Germany
2	Petranova=Lihonova	Germany
3	Erglu	Germany
4	Germany	Germany
5	Eckendorfer Mali	Sweden
6	Murasaki-natane	Japan
7	Isuzu-natane	Japan
8	Target	Canada
9	Nilla=1022	England
10	Szneszowichi	Poland
11	Niederarnbacher	Germany
12	Sv. Gulle	Sweden
13	Mlochowski	Poland
14	Gorczyński	Poland
15	Lembkes malchower	Germany
16	Ölquell	Germany
17	Rapol	Germany
18	R. Crésus	Sweden
19	Major	France
20	Mutsu-natane	Japan
21	Aomori 1	Japan
22	Asahi-natane	Japan
23	Norin 16	Japan
24	Wase-Chousen 618	Japan
25	Wase-Chousen 635	Japan
Alloplasmic line		
26	(chi)Isuzu-natane	
27	(pol)napus × Isuzu/2	

2. MATERIALS AND METHODS

Plant materials

Twenty-seven accessions were selected from our rapeseed collection: Those were (a) 14 varieties carrying the *nap* cytoplasm, (b) one line with the *pol* cytoplasm, and (c) 12 varieties with the normal cytoplasm (Table 1).

Extraction, restriction endonuclease digestion, and gel electrophoresis of mtDNA

Mitochondria were isolated from green leaves of 8-weeks-old plants according to the procedure described by Kemble (1987) with the following modifications; buffer mtA (50 mM Tris-HCl pH 8.0, 3 mM EDTA, 0.1% BSA, 1 mM Me-EtOH and 0.44 M sucrose) and shelf buffer (50 mM Tris-HCl pH 8.0, 20 mM EDTA and 0.6 M sucrose) were used instead of buffers A and B, respectively. The first and third centrifugations were performed at $2500\times g$ instead of $1000\times g$.

MtDNA was purified using CsCl/EtBr buoyant density gradient centrifugation, isopropanol extraction and dialysis. MtDNA samples were digested with the following ten enzymes; *Bam*HI, *Bgl*II, *Eco*RI, *Hind*III, *Kpn*I, *Pst*I, *Pvu*II, *Sal*I, *Sma*I and *Xho*I. Digestion of mtDNA with these enzymes was carried out according to the instructions given by the supplier. Digests were separated by electrophoresis in 0.7% agarose gel.

3. RESULTS

Mitochondrial linear plasmid-like DNA

Mitochondria of many *Brassica* species contain, in addition to the main mitochondrial genome, a linear plasmid-like DNA of approximately 11.3 kb (Palmer et al., 1983b). MtDNAs of all the accessions used in this report were electrophoresed on agarose gels without prior restriction enzyme treatment.

Fig. 1. Electrophoretic patterns of undigested rapeseed mtDNAs. White dot, 11.3 kb linear plasmid band. *, accession number.

Table 2. Classification of the mitochondrial genome types based on fragment patterns of mtDNA treated with 10 restriction enzymes

Accession number	Cultivar	Plasmid	Restriction endonuclease analysis										Mt genome type	Cytoplasm type	Ct genome type
			<i>Bam</i> HI	<i>Bgl</i> III	<i>Eco</i> RI	<i>Hind</i> III	<i>Kpn</i> I	<i>Pst</i> I	<i>Sal</i> I	<i>Sma</i> I	<i>Xho</i> I				
Euplasmic line (cultivar)															
1	Mali	—	2a	2a	2a	2a	2a	2a	2c	2a	2	Ila	N ^{*)}	II **)	
2	Petranova=Lihonova	+	2a	2c	2a	2a	2a	2a	2a	2a	2	Ilc	N	II	
3	Erglu	—	2a	2a	2a	2a	2a	2a	2a	2a	2	Ila	N	II	
4	Germany	—	2a	2a	2a	2a	2a	2a	2a	2a	2	Ila	N	II	
5	Eckendorfer Mali	+	2a	2a	2a	2a	2a	2a	2a	2a	2	Ila	N	II	
6	Murasaki-natane	—	2a	2a	2a	2a	2a	2a	2a	2a	2	Ila	N	II	
7	Isuzu-natane	+	2a	2a	2a	2a	2a	2a	2a	2a	2	Ila	N	II	
8	Target	—	1a	1	1	1a	1	1	1	1	1	Ia	N	I	
9	Nilla=1022	—	1a	1	1	1a	1	1	1	1	1	Ia	N	I	
10	Szneszowichi	+	1a	1	1	1a	1	1	1	1	1	Ia	N	I	
11	Niederarnbacher	+	1a	1	1	1a	1	1	1	1	1	Ia	N	I	
12	Sv. Gulle	—	1a	1	1	1a	1	1	1	1	1	Ia	N	I	
13	Mlochowski	+	1a	1	1	1a	1	1	1	1	1	Ia	S(nap)	I	
14	Gorczanski	—	1a	1	1	1a	1	1	1	1	1	Ia	S(nap)	I	
15	Lembkes malchower	—	1a	1	1	1a	1	1	1	1	1	Ia	S(nap)	I	
16	Ötquell	—	1a	1	1	1a	1	1	1	1	1	Ia	S(nap)	I	
17	Rapol	—	1a	1	1	1a	1	1	1	1	1	Ia	S(nap)	I	
18	R. Crésus	—	1a	1	1	1a	1	1	1	1	1	Ia	S(nap)	I	
19	Major	—	1a	1	1	1a	1	1	1	1	1	Ia	S(nap)	I	
20	Mutsu-natane	—	1a	1	1	1a	1	1	1	1	1	Ia	S(nap)	I	
21	Aomori 1	—	1a	1	1	1a	1	1	1	1	1	Ia	S(nap)	I	
22	Asahi-natane	—	1a	1	1	1b	1	1	1	1	1	Ib	S(nap)	I	
23	Norin 16	—	1a	1	1	1a	1	1	1	1	1	Ia	S(nap)	?	
24	Wase-Chousen 618	—	1a	1	1	1a	1	1	1	1	1	Ia	S(nap)	?	
25	Wase-Chousen 635	—	1b	1	1	1a	1	1	1	1	1	Ic	S(nap)	?	
Alloplasmic line															
26	(chi)Isuzu-natane	+	1a	1	1	1a	1	1	1	1	1	Ia	S(nap)	I	
27	(pol)napus×Isuzu/2	+	2b	2b	2b	2b	2b	2b	2b	2b	2	I1b	S(pol)	?	

*) S(nap), nap cms cytoplasm; S(pol), pol cms cytoplasm; N, normal cytoplasm.

**) Cited from Ohkawa and Uchimiya (1985), and Ohkawa (unpubl.).

Fig. 1 shows the results of a representative experiment.

Of the 27 accessions tested, eight showed a fragment of 11.3 kb below the main genome band (Fig. 1, Table 2).

Restriction endonuclease analysis of mitochondrial DNA

Results are given in Table 2 and Fig. 2. In four accessions, only four endonucleases were used because of a limited amount of mtDNA available.

Fig. 2. Restriction fragment patterns of rapessed mtDNAs and their schematic representations using ten restriction enzymes, *Bam*HI(A), *Bgl*II(B), *Hind*III(C), *Kpn*I(D) and *Sma*I(E). ◁ and ◄, missing and differential fragment, respectively, as compared with the fragments of the subtype a in each major type. P, linear plasmid band. m, molecular size marker (phage lambda DNA fragments digested with *Hind*III).

*Sma*I gave two types of restriction pattern. Of the 23 accessions analyzed, 17 showed pattern 1 and the remaining six pattern 2 (Fig. 2E). Among the accessions showing the *Sma*I-pattern 1, Asahi-natane (acc. no. 22) and Wase-Chousen 635 (acc. no. 25) could be differentiated from other accessions by showing the *Hind*III-pattern 1b and *Bam*HI-pattern 1b, respectively (Fig. 2C and A). Among the accessions with the *Sma*I-pattern 2, (pol) *napus* × *Isuzu*/2 (acc. no. 27) could be distinguished from other accessions by its unique patterns from the digests of all nine enzymes except *Sma*I (Fig. 2A, B, C and D). The digests of four enzymes, *Bgl*III, *Kpn*I, *Pst*I and *Pvu*II, produced the patterns that allowed distinction of Petranova=Lihonova (acc. no. 2) from other accessions with the same *Sma*I-pattern 2 (Fig. 2B and D).

Classification of the mitochondrial genomes based on restriction fragment pattern

Based on the *Sma*I restriction fragment patterns, the mt genomes of the rapeseed accessions were classified into two major types, I and II. Each major type was further divided into three subtypes based on other restriction fragment patterns. As a whole, we could classify the mt genomes of the present materials into six types, Ia, Ib, Ic, IIa, IIb and IIc, as shown in Table 2.

4. DISCUSSION

Intraspecific variation of the mitochondrial genome in rapeseed

Restriction endonuclease analysis of mtDNAs revealed that rapeseed contained two major mt genome types, I and II. The present classification of mt genome is in accord with that of chloroplast genome by Ohkawa and Uchimiya (1985) and Ohkawa (unpublished). The accessions which carried the type I ct genome possessed the type I mt genome, whereas the accessions with the type II ct genome carried the type II mt genome. No accession with the type I ct genome and type II mt genome, or type II ct genome and type I mt genome was found. This association of a specific ct genome with a specific mt genome in rapeseed strongly suggests that the two genomes might have been introduced into rapeseed together when or after *B. napus* was formed. These results are in agreement with the findings of Palmer (1988).

Although Palmer (1988) classified mt genomes of four varieties into two types corresponding to the present major types, we observed small variations within each major mtDNA type, which was divided into three subtypes based on the mtDNA restriction patterns of 27 accessions. However the level of the intraspecific variation of the mt genome was low, because 90% of the type I accessions carried the type Ia mt genome, and type Ib and Ic mt genomes showed only one fragment difference from it.

The present type II mt genome is similar to that of *B. campestris* reported by Palmer (1988). He reported that rapeseed lines with the cytoplasm identical to

that of *B. campestris* had a common breeding history, in which either *B. campestris* or *B. juncea* was used as the maternal parent in hybridization. Shiga (1979) demonstrated that CN (*campestris* × *napus*) as well as NC crosses had been made in breeding Japanese rapeseed varieties. Based on the present data and the informations obtained by Shiga (1979) and Palmer (1988), the cytoplasms with the type II mt genome can be assumed to have originated from *B. campestris*.

Relationship between the mt genome types and male sterility induction

In many plants, recent results suggested that the cms trait is associated with mitochondrial dysfunction (see for a review, Lonsdale, 1987).

Palmer et al. (1983b) reported that mitochondrial plasmid DNA was related to cms induction in rapeseed plants. Although we observed that eight of the 27 accessions studied harbored a 11.3 kb linear plasmid, there was no relationship between the presence of the plasmid DNA and cms induction, supporting the findings of Kemble et al. (1986), who did not find the exclusive association between them either.

In this investigation, we found that the *nap* cms cytoplasm carried either of Ia, Ib and Ic mt genomes and the *pol* cms cytoplasm carried the IIb mt genome.

In earlier studies of crossing experiments (Fu, 1981; Fan et al., 1986), the cms inducing *pol* cytoplasm was maintained by pollinating with the maintainer of the *nap* cms system. Some of the restorers of this system restored male fertility under the presence of *pol* cytoplasm. Therefore, the *pol* cms seems to have a similar developmental bases to the *nap* cms, although their mt genome types were different. The *pol* cytoplasm was classified into type IIb that showed a few fragment differences from type IIa, in which only male-fertile cytoplasms were included. It might be possible to find the mitochondrial gene controlling the cms trait in these fragments, for instance the cms-related fusion gene, *urf13* found in *Xho* 6.6 kb fragment of T-type male sterile maize (Umbeck and Gengenbach, 1983; Dewey et al., 1986).

To our knowledge, many mtDNAs of the cms cytoplasms exhibit altered restriction fragment patterns when compared with its normal counterpart (Dewey et al., 1986; Erickson et al., 1986; Lonsdale, 1987). However, we could not detect the alterations of restriction patterns between the *nap* cms and normal cytoplasm with the Ia mt genome although more than 200 restriction fragments were compared. Therefore the present results suggest the expression of the *nap* cms depends upon the difference in the small part of the mt genome, probably one or a few genes.

The following facts seem noteworthy. 1) The *nap* and *pol* cytoplasms carry a different mt genomes, although they are similar in the responses to the maintainer and the restorer. 2) The *nap* cms cytoplasm and some normal cytoplasms could not be distinguished by restriction analysis of their mtDNAs. These facts suggest that the cms trait in rapeseed (*Brassica napus* L.) is controlled by a very

small part of the mt genome, presumably one or a few genes.

We are greatly indebted to Mr. K. Suenaga, Department of Cell Biology, NIAR, for his helpful discussions and critical reading of the manuscript.

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