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Comparative Macromolecular Analysis of the Cytoplasms of Normal and Cytoplasmic Male Sterile *Brassica napus*

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Summary. Chloroplast (cp) and mitochondrial (mt) compartments of normal (N) and cytoplasmic male sterile (cms) lines of *Brassica napus* have been characterized and compared on the basis of cp and mt DNA restriction enzyme analysis and in vitro protein synthesis by isolated mitochondria. Cytoplasmic male sterility of *B. napus* (rape) comes from cms *Raphanus sativus* (radish) through intergeneric crosses.

Cp DNAs isolated from N and cms lines had distinct restriction patterns with Sal I, Kpn I and Sma I enzymes. The size of the two cp DNAs measured from the restriction patterns was found to be identical and of about 95×10^6 d. N and cms lines of *B. napus* were characterized by specific mt DNAs, as shown from Sal I, Kpn I, Pst I and Xho I cleavage patterns. The small number of well-separated restriction fragments obtained with Sal I enabled us to determine precisely mt DNA sizes. The values of 136.5 and 140.3×10^6 d, obtained from restriction patterns with N and cms DNAs respectively, are smaller than any of those previously obtained from studies on other genera. With molecular hybridization experiments, it was possible to distinguish N and cms lines by the different locations of rRNA genes on the cp and mt DNAs.

Two lines of *B. napus* are characterized by specific mt translation products formed in isolated mitochondria.

Key words: Chloroplast DNAs – Mitochondrial DNAs – Cp and mt r RNA genes – In vitro mitochondrial translation – Maternal inheritance

Introduction

It is well known that the cytoplasm is an important source of heritable variability in eukaryotic organisms. In higher plants, one of the best examples of this type of inheritance is cytoplasmic male sterility, a trait that causes pollen abortion in the anthers. This trait has been extensively used in numerous higher plants for the production of commercial hybrids. There is some biochemical evidence that the mitochondrion, rather than the chloroplast, is the carrier of male-sterility factors in maize, wheat and tobacco. Restriction endonuclease analysis has revealed marked differences between the mitochondrial (mt) DNAs from normal and male-sterile cytoplasms in both maize and wheat, but the corresponding chloroplast (cp) DNAs were indistinguishable (Levings and Pring 1976; Quétier and Vedel 1977). More recently, it has been shown that mitochondria from lines carrying the T, S, C or normal (N) cytoplasms of maize can all be distinguished from one another on the basis of the polypeptides synthesized in vitro by isolated mitochondria (Forde et al. 1980) and low molecular weight mitochondrial DNAs (Kemble et al. 1980). Spontaneous reversion to fertility in S male-sterile cytoplasm of maize has been correlated with the disappearance of the mt plasmid-like DNA's, S-1 and S-2, and the appearance of new mt DNA restriction fragment patterns in the revertants (Levings et al. 1980). In the case of tobacco, the cms trait has been studied in cytoplasmic hybrids obtained from protoplast fusion between two varieties of *N. tabacum*. Restriction enzyme analysis of the cytoplasmic DNAs of different hybrids has shown that only one or the other parental cp DNA was present in the hybrids and their progeny (Belliard et al. 1978) regardless of the phenotype. However, the mt DNAs of hybrids were different from those of the parents and from the mixture of the two, indicating that mt DNA is the cytoplasmic support of cytoplasmic male sterility in tobacco (Belliard et al. 1979).

The rape *Brassica napus* is a natural amphidiploid (AACC genome, $2n = 38$) combining the chromosomal

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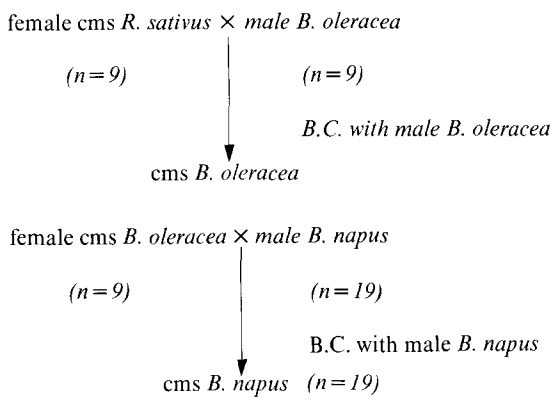
basic sets of *B. oleracea* (CC genome, 2n = 18) and *B. campestris* (AA genome, 2n = 20). Cytoplasmic male sterility of *B. napus* comes from intergeneric crosses involving a Japanese line of cms *Raphanus sativus* (Ogura 1968; Bannerot et al. 1974). Unfortunately, the cms lines of *B. napus* are chlorophyll deficient preventing their use in agronomy. In this study, cp and mt compartments of N and cms lines of *B. napus* have been characterized and compared on the basis of cp and mt DNA restriction enzyme analysis and in vitro protein synthesis by isolated mitochondria.

Materials and Methods

Species Examined

N and cms lines of *B. napus* (Table 1) were grown in a greenhouse of the phytotron in Gif sur Yvette at 22 °C and 16 h daylength. Cms lines of *B. napus* were obtained by sexual crosses involving a cms Japanese radish variety (Ogura 1968; Bannerot et al. 1974):



Isolation of cp and mt DNAs

cp and mt DNAs were isolated from well-expanded leaves as previously described by using CsCl-ethidium bromide gradients (Herrman et al. 1975; Kolodner and Tewari 1975) with some modifications (Vedel et al. 1980). Furthermore, mitochondria were purified by centrifugation in discontinuous sucrose gradients (30%, 40%, 50%, 60% steps in buffer A from Herrman et al. 1975). Mitochondria were removed from the 40%–50% interface, slowly diluted with three volumes of homogenizing medium and recovered (20,000 g, 15 min).

Table 1. *B. napus* lines used in the experiments

Lines	Cytoplasms	Floral phenotype	Agronomic type
Jet 9	*B. napus*	normal	winter oil-seed
R 48	*B. napus*	normal	winter oil-seed
Br Br	*R. sativus*	cms	spring oil-seed
S 82	*R. sativus*	cms	winter oil-seed
S 109	*R. sativus*	cms	winter forage

Jet 9 was provided by Coopérative du Hurepoix, Limours, France; other lines were produced by Station d'Amélioration des Plantes, INRA, Rennes-Le Rheu, France

Isolation and Labeling of cp and mt rRNAs

Organelles, organelle ribosomes and rRNAs were successively isolated as previously described (Whitfeld et al. 1978; Bonen and Gray 1980). Purified rRNAs were labeled with (^{32}P) ATP in vitro following the technique of Maizels (1976) with the following modifications: rRNAs in a solution of 100 mM Tris-HCl pH 9.5 (0.5 µg/µl), were partially hydrolyzed by heating in sealed 20 µl-micropipets at 95 °C, 3 min · 60 µl 10 mM Tris-HCl (pH 7.4), 1 mM spermidine, 0.1 mM EDTA were added to 10 µl of the heated RNA solution and the mixture held at 50 °C for 3 min. Then, to label the 5′ ends, the following was added: 10 µl 500 mM Tris-HCl (pH 9.5), 50 mM dithiothreitol, 100 mM $MgCl_2$, 100 p moles (γ-^{32}P)ATP (Amersham ref. PB 10168, specific activity >2000 Ci/m mol), and several units of T4 polynucleotide kinase (Boehringer Mannheim) to make a final volume of 100 µl. After incubation at 37 °C for 30 min, the reaction was stopped by the addition of 200 µl 2 M ammonium acetate and 50 µg *E. coli* tRNA. The RNAs were precipitated three times with cold ethanol to remove unreacted (γ-^{32}P)ATP. The specific activity of the labeled rRNAs was greater than 10^6 cpm/µg.

DNA Restriction and Agarose Gel Electrophoresis

Two to 4 µg of cp or mt DNA were digested in 30 µl reactions with sufficient enzyme to give complete digestion. The restriction enzymes used were Sal I, Xho I, Pst I, prepared as indicated by Vedel et al. (1980), Sma I (Boehringer Mannheim) and Kpn I (Bethesda Research Laboratories). The restriction fragments were separated by electrophoresis in 0.7% agarose vertical slab gels, 20 or 40 cm long. The procedures of gel staining and ultra violet fluorescence photography have previously been described (Quétier and Vedel 1977).

A mixture of DNA fragments generated from λDNA by Hind III and from λDNA by Hind III + EcoRI (Boehringer Mannheim) was used as a molecular weight standard. Molecular weights of *B. napus* cp and mt DNAs and band multiplicity were determined by the method of Vedel et al. (1980).

DNA/RNA Hybridization

After denaturation, the DNA fragments in gels were transferred to nitrocellulose membrane strips (Schleicher and Schüll BA 85) according to the standard Southern procedure (1975) modified to allow overnight elution from slab gels. For hybridization, DNA strips (0.4 × 20 cm) were soaked with 150 µl of hybridization medium containing 100–500 ng (^{32}P) RNA in 5 × SSC (0.75 M NaCl – 0.075 M sodium citrate), 50% formamide. Strips were dipped in a paraffin oil bath at 42° for 24 h. After hybridization, strips were washed in 2 × SSC successively for 30 min at 30 °C, 30 min at 45 °C, 30 min at 65 °C and 2 h at 65 °C, before being dried and autoradiographed for 1 to 3 days using Ilford X-ray films (Rapid R, type S).

Protein Synthesis by Isolated Mitochondria

Seeds were surface sterilized with sodium hypochlorite and germinated in darkness at 22 °C on sterilized gauze moistened with sterile distilled water. Mitochondria were isolated as mentioned above for mt DNA preparation except that the 4-step sucrose gradients did not contain bovine serum albumin (normally present in buffer A). The band at the 40%/50% sucrose interface was removed and diluted to an osmolarity of 0.6 M

with 0.2 M mannitol, 1 mM EGTA and 10 mM tricine pH 7.2, and the mitochondria were pelleted at 15,000 g for 15 min. Aliquots of the purified mitochondria were resuspended, at about 500 µg protein/250 µl, in the reaction mixture described previously by Forde et al. (1978). A 25 µmol mixture of 19 amino acids (omitting methionine) in 20 µCi L(^{35}S) methionine (Amersham, specific activity > 600 Ci/m mol) were added, energy being provided by the inclusion of 8 mM creatine phosphate, 25 µg creatine phosphokinase and 6 mM ATP. The mitochondria were incubated at 25 °C for 60 min and 5 µl aliquots were removed every 15 min onto Whatman 3 MM paper discs. Radioactivity precipitable by hot trichloroacetic acid was determined according to the procedure of Mans and Novelli (1961). Contribution of bacterial contamination was checked according to Forde et al. (1979). Colonies obtained by plating mitochondrial suspensions were less than 5,000/incubation. Bacterial contribution to the incorporation of (^{35}S) methionine was found to be significant with about 10^5 colonies.

Polyacrylamide Gel Electrophoresis

SDS-PAGE was performed in gel slabs with 12% acrylamide (Laemmli 1970). The samples were dissolved and depolymerized before electrophoresis by a 30 min incubation at 60 °C in 5% SDS, 2% 2-mercaptoethanol, 20% glycerol and 30 mM Tris-HCl (pH 6.8). Gels were stained with Coomassie blue and dried onto Whatman 3 M paper. The dry gels were exposed to Ilford X-ray films for up to 2 weeks. Protein was estimated according to Lowry et al. (1951).

Results

Characterization of cp DNAs Isolated from N and cms Lines of B. napus

Figure 1 shows the electrophoregrams of Sal I, Kpn I, Sma I and Pst I restriction fragments obtained with the cp DNAs isolated from N (R 48) and cms (S 109) lines of *Brassica napus*. The two lines are characterized by specific cp DNA patterns when the DNA is cut with Sal I, Kpn I, and Sma I enzymes. It is noteworthy that N and cms Sal I patterns contain only five and four bands respectively. The two lines give rise to four fragments of identical size. The largest restriction fragment of cms is represented by two fragments in N. In the case of the Kpn I analysis, the N and cms patterns are characterized by nine common bands and differ only by the presence of one fragment of 2.65×10^6 d in the N pattern instead of by two fragments of 2.7×10^6 d. Sma I cleavage of N and cms cp DNAs leads to eight and nine bands respectively with seven common bands (Table 2). Homology of differing bands can also easily be established in this case. Identical Pst I patterns are observed with both cp DNAs. It has been verified that the two N lines described in Table 1 have identical cp

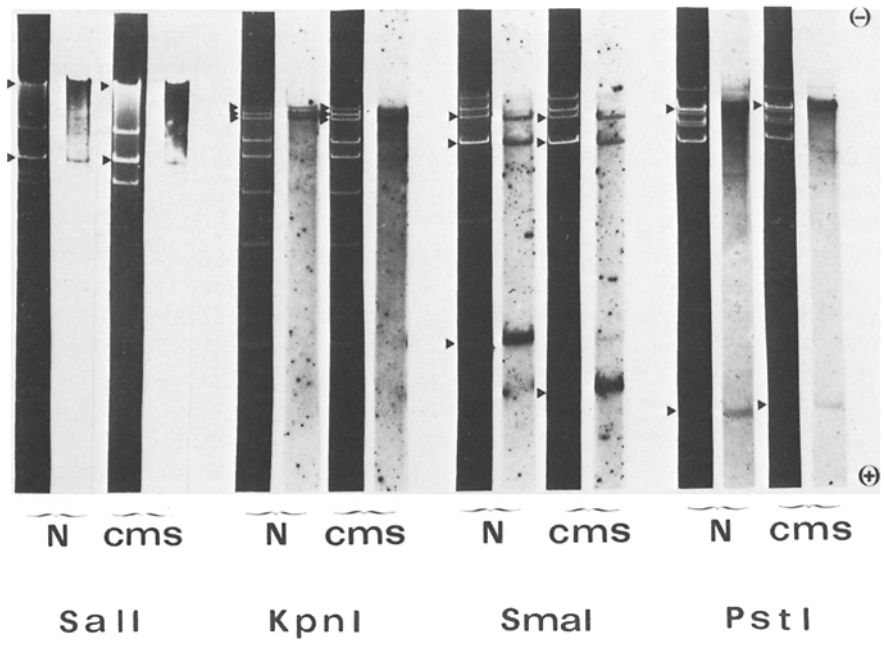


Fig. 1. Sal I, Kpn I, Sma I and Pst I restriction patterns of cp DNAs from N (R 48) and cms (S 109) *B. napus* and autoradiographs of molecular hybridization between N and cms radioactively labeled cp rRNAs and corresponding cp DNA patterns. The DNA fragments were separated by electrophoresis on 0.7% agarose slab gels, denatured, transferred to nitrocellulose paper by Southern blotting and the filters incubated with the radioactive RNA probes. On the left are the respective gel patterns, and on the right, the corresponding autoradiographs

Table 2. Molecular weights ($\times 10^6$ d) of normal and cms *B. napus* cp DNA fragments produced by Sal I, Kpn I, Sma I and Pst I restriction enzymes

Sal I		Kpn I		Sma I		Pst I
N	cms	N	cms	N	cms	N and cms
–	62.4[a]	19.5[a]	19.5[a]	25	25	17.8
56[a]	–	17.4[a]	17.4[a]	17.8	17.8	13.2[a]
11.3	11.3	16.6[a]	16.6[a]	15.9[a]	15.9[a]	12.6
7.7 (2)	7.7 (2)	12.6	12.6	12.1[a] (2)	12.1[a] (2)	11.3
6.4	–	10.3	10.3	5.7	5.7	10.8
6.1	6.1	7.1	7.1	2.55[a] (2)	–	8.6 (2)
		4.6	4.6	–	2.0[a] (2)	3.3
		3.0	3.0	1.0	1.0	1.4[a]
		2.7	2.7 (2)	0.65	0.65	1.35
		2.65	–	–	0.55 (2)	1.05
						0.8
						0.55
						0.5
						0.35
Total 95.2		96.2		95.35		92.25

A mixture of DNA fragments generated from λ DNA by Hind III and from λ DNA by Hind III + EcoRI was used as a molecular weight standard in all instances. Fragments were measured on 0.7% agarose gels. Numbers in parentheses refer to molar concentrations greater than one, to the nearest whole number

[a] Indicate rDNA fragments

DNAs. Identical patterns have also been found among cp DNAs of cms lines as well as among these cp DNAs and the cp DNA of the cms lines of the Japanese radish.

The total molecular weight of cp DNAs was obtained by the addition of the molecular weights of all the restriction fragments present on a given pattern, as shown in Table 2. The sizes of N and cms cp DNAs appear identical when determined with comparative restriction patterns. The values range from 92.25×10^6 d with Pst I to 96.2×10^6 d with Kpn I. In these determinations the band multiplicity was taken into account and determined as indicated previously (Vedel et al. 1980). Complete homologies occur in the distribution of the double bands in the Sal I (7.7×10^6 fragment) and Pst I (8.6×10^6 d fragment) electrophoregrams, of the two types of cp DNA. By the distribution of the double bands in the Sma I and Kpn I restriction patterns, it is possible to distinguish the two lines.

Molecular hybridization of total chloroplast ribosomal RNA (rRNA) with N and cm cp DNA restriction fragments results in the labeling of the same or similarly sized fragments in all cases except the hybridizations to Sma I fragments (see autoradiograms in Fig. 1 and Table 2). Hybridization occurs with the 2.55×10^6 d Sma I double band in N but to the 2.0×10^6 d Sma double band in cms. This provides an excellent marker by which to distinguish the two types of cp DNA, and supports the notion that the 2.55×10^6 d fragments in N are homologous to the $2.0 + 0.55 \times 10^6$ d fragments of cms. Hybridization of 23 S, 16 S and 5 S fractions of cp rRNA with cp DNA restriction fragments led to autoradiograms that appeared identical to those obtained with total cp rRNA, indicating that degradation occurred during the fractionation procedure. This degradation could be related to the observation that *B. napus* chloroplasts have been found to be very fragile despite mild homogenisation of the leaves.

Characterization of mt DNAs Isolated from N and cms Lines of B. napus

Comparison of the Sal I, Kpn I, Pst I and Xho I patterns of the mt DNAs isolated from N and cms *B. napus* revealed that the two plant types contain distinct mt DNAs (Fig. 2). We have found identical mt DNAs inside each type as in the case of cp DNAs. The mt DNA isolated from the cms Japanese radish was analyzed with the Sal I enzyme only. The pattern was identical to the corresponding one in cms *B. napus*. The mt DNA patterns contain a greater number of restriction fragments than corresponding cp DNA patterns. Electrophoregrams of Sal I mt DNA fragments of N and cms *B. napus* are characterized by 21 and 23 fragments respectively. As shown in Table 3, N and cms mt DNAs possess seven and nine specific Sal I bands respectively and fourteen in common. The small number of Sal I mt DNA bands obtained with *B. napus* (in comparison to other higher plants) and their good electrophoretic separation enable us to measure both fragment size and

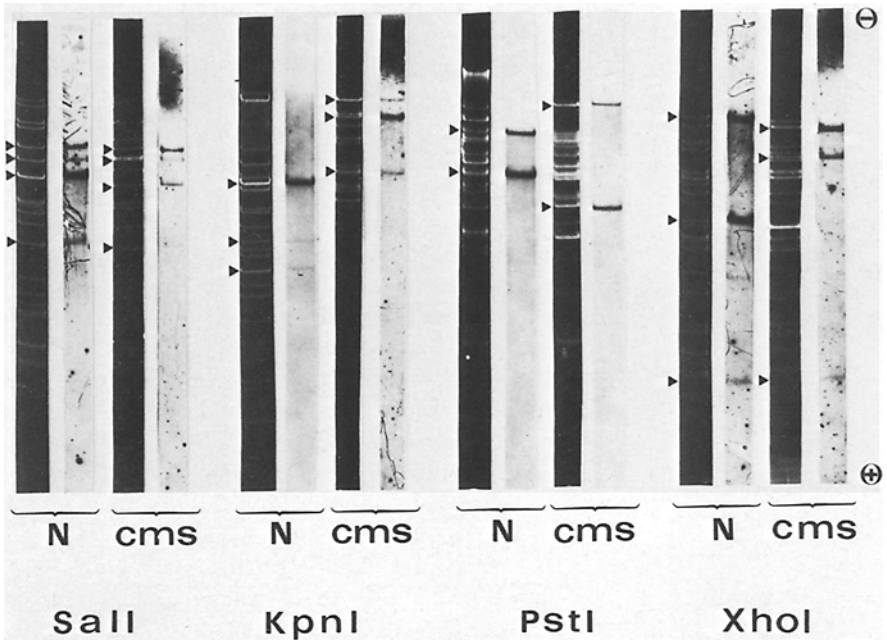


Fig. 2. Sal I, Kpn I, Pst I and Xho I restriction patterns of mt DNAs from N (R 48) and cms (S 109) *B. napus* and autoradiographs of molecular hybridization between N and cms radioactively labeled mt rRNAs and corresponding mt DNA patterns. The DNA fragments were separated by electrophoresis on 0.7% agarose slab gels, denatured, transferred to nitrocellulose paper by Southern blotting and the filters incubated with the radioactive RNA probes. On the left are the respective gel patterns, and on the right, the corresponding autoradiographs

band multiplicity. The size of N and cms mt DNAs, determined by summing the molecular weights of all the Sal I fragments, ranged from 136 to 140×10^6 d (Table 3).

Differences between the N and cms lines of *B. napus* is also evidenced in the autoradiograms of Figure 2 by comparing the location of the mitochondrial rRNA genes on the fragments obtained with the different restriction enzymes. Total mitochondrial rRNA hybridizes with four Sal I, three Kpn I, three Xho I and two Pst I mt DNA fragments in both cases. As shown in Table 4, N and cms *B. napus* rRNA genes are found on mt DNA restriction fragments of very different sizes when the two lines are compared. Indeed, hybridization with fragments of identical size occurs only with two Sal I and one Xho I fragments. Individual mitochondrial rRNA species (26 S, 18 S, 5 S) purified from N and cms *B. napus* hybridized with the same mt DNA fragments as total mt rRNA.

Protein Synthesis by Isolated Mitochondria from N and cms B. napus

Mitochondria were prepared from either cotyledons or hypocotyls of six day-old etiolated seedlings and allowed to incorporate (^{35}S) methionine by the procedure described in Materials and Methods. We have observed that incorporation of (^{35}S) methionine into hot trichlo-

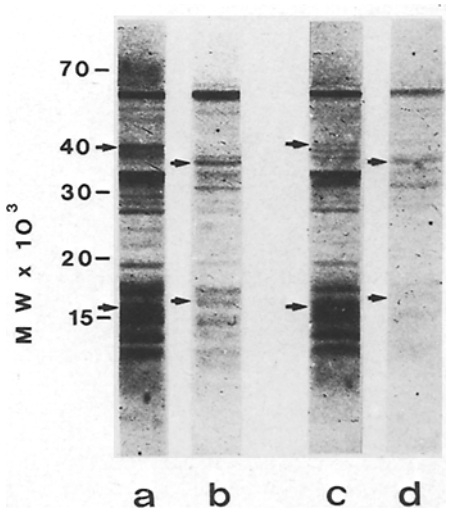


Fig. 3a–d. Electrophoresis of polypeptides synthesized by mitochondria from: **a** normal cotyledons; **b** cotyledons of male-sterile plants; **c** normal hypocotyls; **d** hypocotyls of male-sterile plants of *B. napus*. Jet 9 and S 109 were respectively used as N and cms lines. Mitochondria were isolated and incubated (200 µg protein in each case) for 60 min in 250 µl of medium containing (^{35}S) methionine and an energy-generating system according to Materials and Methods. Identical aliquots were fractionated by SDS-12% polyacrylamide slab gel electrophoresis. Gels were stained, dried and submitted to autoradiography. Serum albumin, ovalbumin, chymotrypsinogen and RNase were used as M. W. markers. Arrows indicate the polypeptides not common to both lines

Table 3. Molecular weights ($\times 10^6$) of normal and cms *B. napus* mt DNA fragments produced by Sal I restriction enzyme

Line of *B. napus*	N	cms
Number of bands	21	23
	18.0	–
	–	17.5
	16.5	16.5
	12.5	–
	11.0	11.0
	–	9.0
	8.5	–
	–	8.0
	7.5	7.5 (2)
	–	7.3
	–	6.5
	6.1 (2)	–
	–	5.8
	5.1 (2)	5.1
	5.0	5.0
	4.8	–
	4.4	4.4
	3.9	3.9
	–	3.35
	3.1	3.1
	–	3.0
	–	2.9
	2.8	2.8
	2.6	2.6
	2.5	2.5
	2.4	–
	2.0 (2)	2.0
	1.8 (2)	1.8
	0.65	–
	0.57	0.57
Total	136.72	139.62

Table 4. Size of rDNA fragments ($\times 10^6$ d) from normal and cms *B. napus* mt DNAs as measured from Sal I, Kpn I, Pst I and Xho I restriction enzyme patterns

	Sal I		Kpn I		Pst I		Xho I	
	N	cms	N	cms	N	cms	N	cms
	8.5	8.0	5.4	14.0	8.0	12.7	8.0	7.0
rDNA	7.5	7.5	3.65	9.9	5.6	4.3	3.6	5.5
Fragment	6.1	5.8	3.0	5.6			1.13	1.13
Size	3.9	3.9						

roacetic acid precipitable material reaches a plateau at about 60 min for N and cms plants and is irrespective of their origin as to organ. Cycloheximide (50 µg/ml) did not perceptibly modify this incorporation. Chloramphenicol (1 mg/ml), known to be a strong inhibitor of the biogenesis of bacterial and mitochondrial proteins (Gillham 1978), inhibited it completely.

The labeled products synthesized by isolated mitochondria were fractionated by polyacrylamide gel electrophoresis and detected by autoradiography. Profiles of the polypeptides synthesized by mitochondria from N and cms cotyledons and by mitochondria from N and cms hypocotyls are shown in Figure 3. About 20 polypeptides were present in each case. Identical products appear to be synthesized by mitochondria isolated from either cotyledons or hypocotyls for a given type of plant. However, quantitative variations occur in favour of cotyledons whatever the polypeptide (compare in Fig. 3a with c and b with d). Comparison of the polypeptides synthesized in vitro by N and cms mitochondria reveals in addition to some quantitative variations, that the polypeptides of 40×10^3 d and 16×10^3 d apparently are synthesized only by N mitochondria (arrows in Fig. 3, lanes a and c) and the polypeptides of 37×10^3 d and 17×10^3 d only by cms mitochondria (arrows in Fig. 3, lanes b and d). Constituent polypeptides of N and cms mitochondria could not be distinguished by staining the gels with Coomassie blue.

Discussion

Normal and cytoplasmic male sterile lines of *B. napus* have been distinguished by restriction analysis of cp and mt DNA, location of rRNA genes and in vitro protein synthesis products of isolated mitochondria.

Sal I, Kpn I and Sma I cp DNA patterns of the N lines are distinct from the corresponding cms ones. Sal I, Kpn I, Pst I and Xho I mt DNA patterns obtained with the N plants are also different from the corresponding ones of the cms plants. The observation that Sal I patterns of cms cp DNAs and cms mt DNAs appeared identical to the corresponding patterns of the cms Japanese radish, used to transfer the cms trait into *B. napus,* indicates that cp and mt DNAs in the cms lines of *B. napus* originate from the cms *B. oleracea* and inherited maternally thereafter.

The sizes of the two types of cp DNAs appear very similar, about 95×10^6 d in size. In the case of the mt DNAs, the sizes observed from the Sal I patterns were found to be 136.5 and 140.3×10^6 d in N and cms lines respectively. This difference is too small to be significant. More important is the observation that the size value of the radish mt DNA is very close to the values found recently with mt DNAs from several other *Brassica* species (Lebacq and Vedel 1981). Indeed, the mitochondrial genomes of the Cruciferae are smaller than all those published for other families by measurements from Sal I restriction patterns. This represents a very valuable advantage to (1) study mitochondrial gene mapping and genome organization in higher

plants (2) and may shed light on the contradictory results published recently (Spruill et al. 1980; Dale 1981).

N and cms *B. napus* have been distinguished by different locations of cp rRNA genes on Sma I cp DNA fragments. Cp rRNA hybridizes with three Sma I fragments, 15.9, 12.1 and 2.55 (N) or 2.0 (cms) $\times 10^6$ d in size and with three Kpn I fragments, 19.5, 17.4 and 16.6×10^6 d in size (Table 2). These results suggest that at least two copies of the cp rRNA genes are present per chloroplast DNA molecule in *B. napus*, as in many other higher plants analyzed (Whitfeld et al. 1978) and unlike in *Vicia faba* (Koller and Delius 1980) and other leguminosae (Kolodner and Tewari 1979). Hybridization of cp rRNA genes of N and cms *B. napus* with bimolar fragments suggests furthermore that cp rRNA genes are located on an inverted repeat region of cp DNA (Bedbrook and Kolodner 1979). N and cms *B. napus* are characterized by specific hybridization patterns between mt rRNAs and mt DNA fragments released by each of the four enzymes used. In each case, the number of mt DNA fragments bearing mt rRNA genes is identical in both lines. This number has been found to be higher with wheat mt DNA (Bonen and Gray 1980) cleaved with the same enzymes.

The two lines of *B. napus* have been characterized by their mitochondrial translation products formed by isolated mitochondria in the presence of (^{35}S) methionine. Autoradiography after electrophoresis of the labeled polypeptides has revealed (1) the presence in both cases of about 20 polypeptides, as observed with maize mitochondria (Forde et al. 1978, 1979); (2) increased labeling in the N patterns in Figure 3 indicating that in vitro translation is more efficient with N mitochondria than with cms ones for six-days old etiolated seedling; (3) the labeling in both cases of specific polypeptides. That the cms specific products are synthesized by the cms radish mitochondria remains to be demonstrated.

In this paper, we have analyzed comparatively some cytoplasmic macromolecules of N and cms *B. napus*. The cms plants present two maternally inherited traits, chlorophyll deficiency and cytoplasmic male sterility resulting from nucleo-cytoplasmic interactions. Our results enable us to distinguish easily between cp and mt DNAs of N and cms *B. napus*. Organelle DNAs will be useful cytoplasmic markers in attempts to dissociate the chlorophyll deficiency and cytoplasmic male sterility by somatic hybridization.

Acknowledgement

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