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A rapid, single leaf, nucleic acid assay for determining the cytoplasmic organelle complement of rapeseed and related *Brassica* species

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Summary. An assay is described whereby *Eco* RI restriction fragment length polymorphisms of mitochondrial and chloroplast DNAs can definitively identify cytoplasms of interest in *Brassica* crop development. Restrictable mitochondrial and chloroplast DNA is extracted from as little as 2–3 g and 0.5 g leaf tissue, respectively, and the donor plants are able to continue to develop in a normal manner. An unknown cytoplasm can be identified in three days, which is a considerable saving in time and labor compared to the several years required by traditional methods. The assay is very inexpensive and should be established as a routine procedure in laboratories involved in sexual or somatic *Brassica* hybrid production.

Key words: *Brassica* – Mitochondrial DNA (mtDNA) – Chloroplast DNA (cpDNA) – Cytoplasmic male sterility (*cms*) – RFLP

Introduction

The quality and favorable price of rapeseed oil has resulted in a rapid expansion of the world acreage of this crop, especially in Europe, Canada and the Far East, particularly China. This increase in popularity is, in part, due to the development of high quality varieties termed 'canola' (*Brassica napus* or *Brassica campestris*) which are defined as possessing less than 5% erucic acid in the seed oil and less than 30 μmoles glucosinolates per gram of oil-free seed meal (Stefansson and Kondra 1975). The granting of GRAS (Generally Regarded as Safe) status by the FDA in the U.S.A. in 1985 will probably also make canola a major crop in that country.

We (Barsby et al. 1987) and others (Pelletier et al. 1983) have applied protoplast fusion techniques to further develop the crop. This has involved, in our case, constructing cybrids which combine a *B. napus* nucleus with mitochondria from cytoplasmically male sterile (*cms*) sources such as *pol* and *nap* cytoplasms and chloroplasts from *B. campestris* which encode resistance to the triazine group of herbicides. The only method of definitively characterizing the desired cybrids from all the other possible organelle combinations was to develop a mitochondrial DNA (mtDNA) and chloroplast DNA (cpDNA) assay system which could differentiate among all the possible combinations.

Reported here is such an assay which is rapid, inexpensive and non-destructive since it removes only one or two leaves and the plants are able to develop and mature in a normal manner.

In addition to the cybrid characterization application, this restriction fragment length polymorphism (RFLP) assay can also be used to determine the cytoplasmic organelle component of any rapeseed and related *Brassica* species produced by conventional plant breeding techniques. At present, the method employed to confirm the presence of a particular *cms* cytoplasm or to identify an unknown *cms* cytoplasm is a very labor-intensive backcrossing program involving many different fertility restorer and maintainer lines. This procedure is also extremely time consuming because it can take several years to obtain the result. This is especially true in the case of winter varieties where a vernalization period is required. The assay described here, which has been established as a routine screening procedure in our laboratory, can definitively identify both cytoplasmic organelles in just three days.

Materials and methods

Plant material

Plants of any age, grown under any condition, can be utilized in the assay. The specific lines used were (1) *B. napus* spring

Table 1. Number of *B. napus, B. campestris* and *B. juncea* lines analysed carrying natural and alien cytoplasms

Nuclear genome	Cytoplasmic genomes						
	nap	*cam*	*pol*	*ogu*	*jun*	*car*	*mur*
B. napus	45	30	9	7	1	1	4
B. campestris	0	6	1	1	0	0	0
B. juncea	0	0	1	0	5	0	1

and winter varieties carrying *nap* (from *B. napus*), *cam* (from *B. campestris*), *pol* (*Polima* cytoplasm introduced from China via Poland), *ogu* (*Ogura* cytoplasm from a Japanese radish variety; Ogura 1968), *jun* (from *B. juncea*), *car* (from *B. carinata*), *mur* (from *Diplotaxis muralis*) cytoplasms, (2) *B. campestris* varieties carrying *cam, pol* and *ogu* cytoplasms, (3) *B. juncea* varieties carrying *jun, mur* and *pol* cytoplasms, (4) *B. napus* somatic cybrids constructed by protoplast fusions carrying *nap, cam* and *pol* cytoplasms (Barsby et al. 1987) and (5) the following species carrying their own naturally-occurring cytoplasms; *B. carinata, B. nigra* (*nig* cytoplasm), *Raphanus sativus* (*rap* cytoplasm, see Shiga 1980), *Sinapis alba, B. alboglabra, B. amplexicaulis, B. barrelieri, B. certica, B. deflexa, B. elongate, B. fruticulosa, B. gravinae, B. insularis, B. maurorum, B. rupestris, B. spinescens* and *B. tournefortii.*

Table 1 indicates the number of different lines analysed in categories (1) (2) and (3) above. To date, 26 somatic cybrids have been identified in category (4).

Extraction of mtDNA

The method is a modification of the maize mtDNA extraction procedure described by Kemble et al. (1980). All procedures were performed at 4 °C unless otherwise stated. All centrifugation steps utilized 50 ml tubes in a SS34 rotor and Sorval RC-5B centrifuge. Centrifugation pellets were resuspended with a small (no. 4) artists paintbrush. Fewer organelles were broken using the paintbrush than by other methods (eg., continuous passages through a pipette).

Routinely 10–15 g of leaf tissue was used but the procedure can be performed on as little as 2–3 g, ie. less than one leaf. The tissue was homogenized in either a mortar and pestle or a Waring blender (2×5 second pulses at high speed) containing 70 ml of Buffer A (10 mM TES pH 7.2, 0.5 M Mannitol, 1 mM EGTA, 0.2% BSA and 0.05% cysteine). A greater yield of intact mitochondria was obtained with the mortar and pestle. The homogenate was filtered through four layers of cheesecloth, one layer of Miracloth (Calbiochem Inc.) and centrifuged at 1,000×g for 10 min. Mitochondria in the supernatant were concentrated by pelletting at 17,000×g for 10 min, resuspended in 10 ml Buffer A and again centrifuged at 1,000×g for 10 min to remove remaining chloroplasts and nuclei. 1 M magnesium chloride and 10 mg/ml freshly prepared DNase solution were added to the supernatant to give final concentrations of 10 mM and 10 µg/g fresh weight leaf tissue respectively. After incubation at 4 °C for 1 h, mitochondria were centrifuged through 20 ml Buffer B (10 mM TES pH 7.2, 20 mM EDTA and 0.6 M sucrose) at 17,000×g for 20 min. The pellet was resuspended in 10 ml Buffer B, recentrifuged at 17,000×g for 10 min and the final pellet lysed and incubated, with gentle agitation, at 37 °C for 1 h in Buffer C (50 mM Tris-HCl pH 8.0, 10 mM EDTA, 2% sarkosyl and 0.012% Proteinase K). The lysate was made up to 0.2 M ammonium acetate, and the mtDNA purified by three cycles of phenol-chloroform extractions at room temperature prior to ethanol precipitation at −20 °C overnight or at −80 °C for 2 h. Again at room temperature, the DNA precipitate was concentrated by centrifugation, washed two times with 70% ethanol and resuspended in sterile distilled water. mtDNA samples were either immediately subjected to restriction enzyme digestions or stored at −20 °C until required.

Extraction of cpDNA

The method is a modification of those described for potato (Kemble and Shepard 1984) and *Atriplex triangularis* (Palmer 1982). All procedures were performed at 4 °C; all centrifugation steps, unless otherwise stated, involved a SS34 rotor in a Sorval RC-5B centrifuge and all centrifugation pellets were resuspended with a no. 4 paintbrush.

Routinely 5 g of leaf tissue was homogenized in a Waring blender for 2×5 s pulses at highspeed with 70 ml Buffer D (equivalent to Palmer's isolation buffer; 0.35 M sorbitol, 50 mM Tris-HCl pH 8.0, 5 mM EDTA, 0.1% BSA, 15 mM 2-mercaptoethanol, 1 mM spermine and 1 mM spermidine). However, as little as 0.5 g of a leaf can be successfully used in this procedure. The homogenate was filtered through 4 layers of cheesecloth and one layer of Miracloth prior to centrifugation at 1,000×g for 10 min. The pellet was resuspended in 10 ml Buffer E (equivalent to Palmer's wash buffer; 0.35 M sorbitol, 50 mM Tris-HCl pH 8.0, 25 mM EDTA, 1 mM spermine and 1 mM spermidine), again centrifuged at 1,000×g for 10 min, resuspended in 9.5 ml Buffer E and layered onto a step gradient consisting of 7 ml 30% sucrose and 18 ml 60% sucrose both in Buffer E. Centrifugation at 25,000 rpm for 40 min in a Beckman SW27 swing-out rotor and L8 ultracentrifuge resulted in purified chloroplasts collecting at the 30%:60% interface. The band was removed with a pipette, diluted with 30 ml Buffer E and centrifuged at 1,500×g for 15 min. The final pellet was lysed in Buffer C and cpDNA purified exactly as described for mtDNA except that only two cycles of phenol-chloroform extractions were performed.

Restriction enzyme analysis and gel electrophoresis

Restriction enzyme fragmentations of mtDNAs and cpDNAs were performed using an excess of enzyme (on a specific activity basis) for 6 h or, more routinely, overnight. Horizontal submarine agarose gels, usually 0.7% or 1% in concentration, were prepared, electrophoresed at 2.4 V/cm for 16 h, stained and photographed using a 302 nm UV transilluminator as previously described (Kemble et al. 1980).

Results

Following the extraction methods described above, mtDNA and cpDNA was obtained from as little as 2 g and 0.5 g of *Brassica* leaf tissue, respectively. The DNA preparations were of sufficient quality to be fragmented by restriction enzymes and exhibit clear patterns on agarose gels after electrophoresis.

Many different restriction enzymes were employed in an attempt to find one, or a series, which could definitively differentiate between both the mitochondrial and chloroplast genomes of all the *Brassica* species in which we were interested. The enzyme which best fulfilled these criteria was determined to be *Eco* RI. This enzyme was capable of clearly differentiating

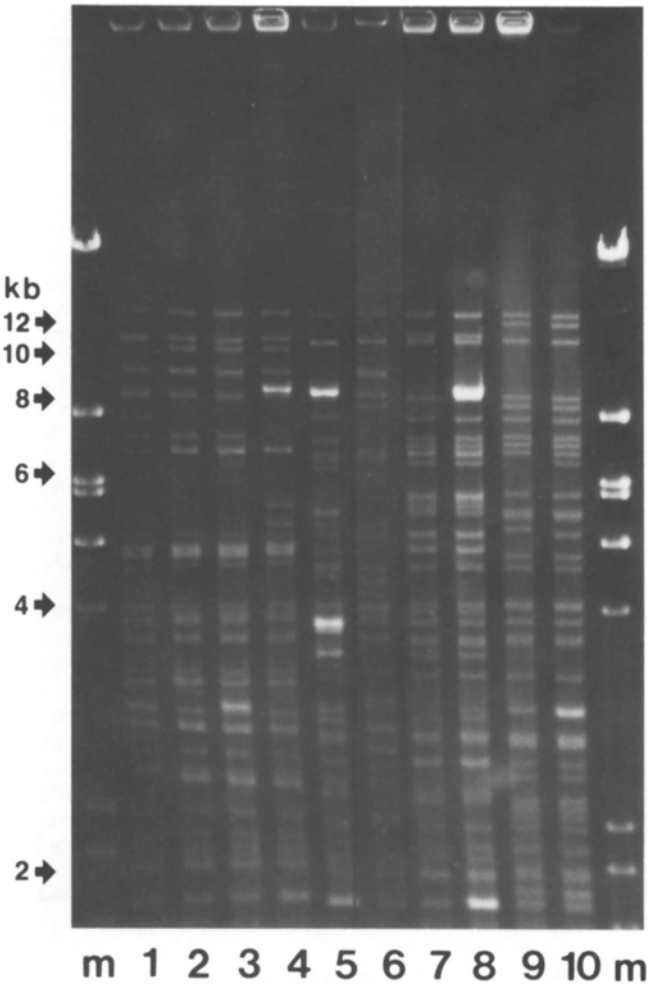

Fig. 1. Characteristic *Eco* RI mtDNA restriction fragment patterns on 1% agarose gel of lane 1: *nap* (from *B. napus* cv. 'Chisaya-natane'); lane 2: *cam* (*B. napus* cv. 'Liné' with *cam* cytoplasm); lane 3: *jun* (*B. juncea* cv. 'Domo'); lane 4: *pol* (*B. napus* cv. 'Karat' with *pol* cytoplasm); lane 5: *ogu* (*B. napus* cv. 'Karat' with *ogu* cytoplasm); lane 6, *rap* (*R. sativus*); lane 7: *mur* (*B. napus* cv. 'Karat' with *mur* cytoplasm); lane 8: *mur* (*B. juncea* cv. 'ZEM' with *mur* cytoplasm); lane 9: *car* (*B. carinata*) and lane 10: *nig* (*B. nigra*). Lanes m contain size markers fragments produced by independent digestions of lambda DNA with *Eco* RI and *Hae* III

between the mitochondrial genomes of *nap, pol, ogu, rap, mur, car* and *nig* cytoplasms (Fig. 1, lanes 1 and 4–10, respectively). Although the *cam* and *jun* mtDNA patterns (lanes 2 and 3, respectively) were identical, they were very distinct from those of the other cytoplasms.

The strongly fluorescent fragments of approximately 8.3 kb observed in the mtDNA patterns of *pol, ogu* and *mur* (Fig. 1, lanes 4, 5 and 8, respectively) represent the largest *Eco* RI fragment of the 11.3 kb linear mitochondrial plasmid which exists in some *Brassica* lines (Palmer et al. 1983). Although this latter publication suggested that the size of this *Eco* RI fragment was 7.8 kb, it routinely migrated to a position of 8.3 kb in our electrophoresis system. The presence or absence of the mitochondrial plasmid and its relationship to *cms* has been reported (Kemble et al. 1986). Plasmid presence or absence can also be useful in differentiating between *cam* and *jun* mtDNA. The plasmid was not observed in the *jun* cytoplasm lines analysed (5 with *B. juncea* nucleus and one with *B. napus* nucleus) but was present in 5 out of 6 *B. campestris* lines carrying *cam* cytoplasm and in 15 out of 30 *B. napus* lines carrying *cam* cytoplasm. The *cam* mtDNA sample shown in lane 2 is from *Brassica napus* cv. 'Liné' which does not possess the plasmid. The highly fluorescent band of 3.8 kb visible in lane 5 is RNA which copurifies with mitochondria extracted from certain plants (Kemble et al. 1986).

Eco RI also produced clear differences between cpDNAs from *nap, pol, ogu, rap, mur, car* and *nig* cytoplasms (Fig. 2, lanes 1, 2 and 5–10, respectively). Most of these cpDNA differences involved fewer fragments than the mtDNA differences. For example, *ogu* (lane 6) and *rap* (lane 7) cpDNAs were differentiated by one fragment size polymorphism (a 1.45 kb *ogu* fragment was present as a 1.5 kb fragment in *rap*) and by the absence of a 2.5 kb fragment in the latter. *Pol* cpDNA differed from *cam* and *jun* cpDNAs by the absence, or severe diminution, of a single fragment of 3.3 kb (lanes 5, 3 and 4, respectively). *Car* and *nig* cpDNA patterns (lanes 9 and 10, respectively) were similar except that the former possessed a fragment of 5.4 kb which was not present in the latter and, conversely, *nig* possessed a fragment of 8.1 kb which was absent in *car*.

Two different *Eco* RI *nap* cpDNA patterns were evident (Fig. 2). Lane 1 contains cpDNA from the normal male fertile *nap* cytoplasm and lane 2 contains cpDNA from Shiga's (1980) *cms nap* cytoplasm. Both cytoplasms were carried in isogenic *B. napus* cv. 'Regent' plants. Shiga's cpDNA possessed a stoichiometric fragment of 3.2 kb which was not observed in the cpDNA pattern from male fertile *nap* cytoplasm. The latter, however, exhibited a sub-stoichiometric fragment of 3.3 kb which was not present in Shiga's cpDNA. This result is surprising because the *cms* trait is considered to be encoded on mtDNA, not cpDNA. However, no restriction fragment differences were observed between male fertile and *cms nap* mtDNA.

As with mtDNA, the two most similar *Eco* RI cpDNA patterns were exhibited by *cam* and *jun* cytoplasms (Fig. 2, lanes 3 and 4, respectively). *Cam* cpDNA possessed a 2.5 kb fragment which *jun* did not and *jun* possessed a substoichiometric fragment of 4.1 kb which was absent in *cam* cpDNA. However, as will be discussed later, both cpDNA patterns varied

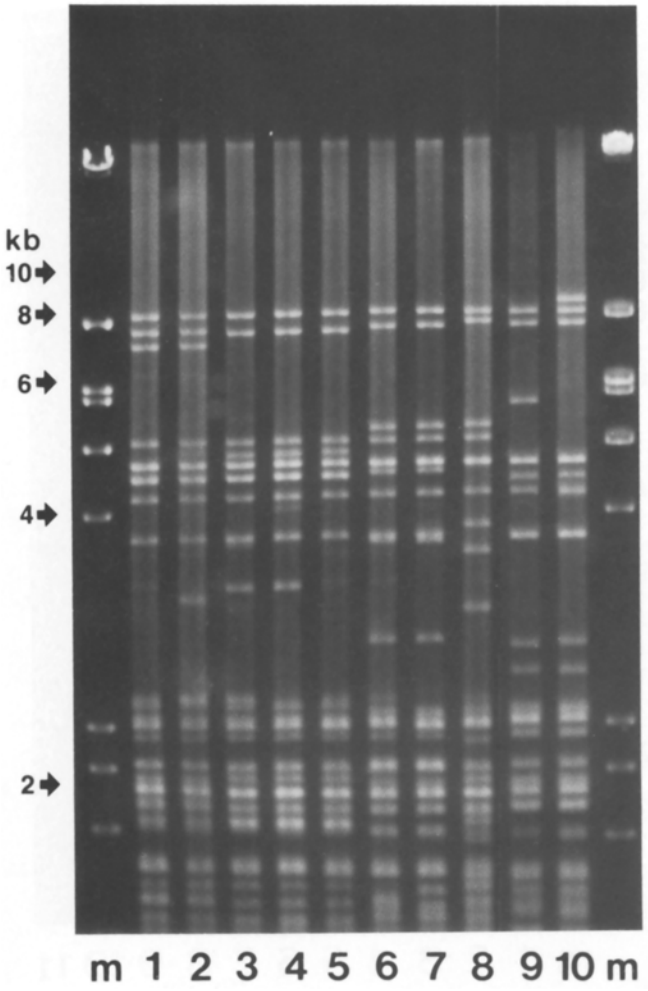

Fig. 2. Characteristic *Eco* RI cpDNA restriction fragment patterns on 1% agarose gel of lane 1: *nap* (from *B. napus* cv. 'Regent'); lane 2: *nap* (Shiga's *cms B. napus* line); lane 3: *cam* (*B. campestris* cv. 'Tobin'); lane 4: *jun* (*B. juncea* cv. 'ZEM'); lane 5: *pol* (*B. napus* cv. 'Westar' with *pol* cytoplasm); lane 6: *ogu* (*B. napus* cv. 'Regent' with *ogu* cytoplasm); lane 7: *rap* (*R. sativus*); lane 8: *mur* (*B. napus* cv. 'Karat' with *mur* cytoplasm); lane 9: *car* (*B. carinata*) and lane 10: *nig* (*B. nigra*). Lanes m as in Fig. 1

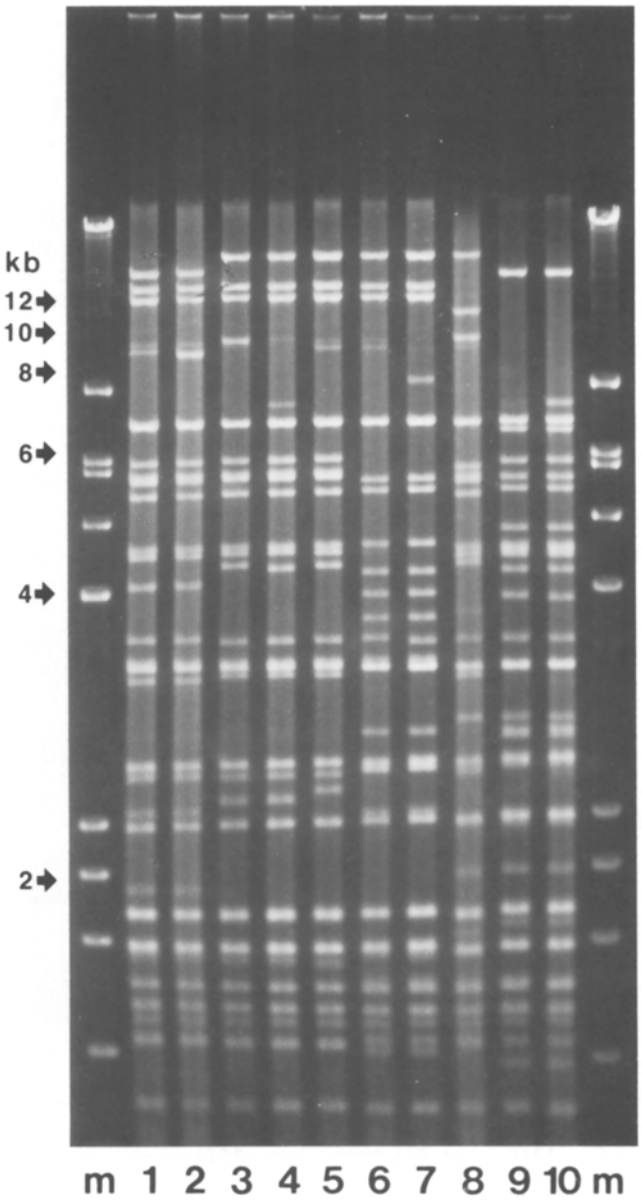

Fig. 3. Characteristic *Bcl* I cpDNA restriction fragments patterns on 1% agarose gel of lane 1: *nap;* lane 2: *cms nap;* lane 3: *cam;* lane 4: *jun;* lane 5: *pol;* lane 6: *ogu;* lane 7: *rap;* lane 8: *mur;* lane 9: *car;* lane 10: *nig.* Lanes m as in Fig. 1

depending on the nuclear background of the plants from which it was extracted.

In addition to *Eco* RI, *Bcl* I was useful in differentiating between *Brassica* cpDNAs. Characteristic *Bcl* I patterns are shown in Fig. 3. Again, *ogu* and *rap* cpDNAs (lanes 6 and 7, repectively) were very similar and could only be differentiated by sub-stoichiometric fragments (7.7 kb fragment present in *rap,* 9.6 and 9.3 kb fragments present in *ogu*). *Nig* (lane 10) differed from *car* (lane 9) by the addition of a single 7.0 kb fragment. Male fertile (lane 1) and *cms* (lane 2) *nap* cpDNA differed only in the relative stoichiometry of one fragment (the 9.0 kb fragment was in stoichiometric amounts in the *cms* version but in sub-stoichiometric amounts in the male fertile type). *Cam* (lane 3), *jun* (lane 4) and *pol* (lane 5) cpDNAs differed with respect to sub-stoichiometric fragments of 9.6, 9.3 and 7.0 kb but could be clearly differentiated by two subtle stoichiometric RFLPs. *Cam* cpDNA possessed fragments of 4.3 and 2.4 kb, *jun* possessed fragments of 4.2 and 2.4 kb and *pol* possessed fragments of 4.3 and 2.5 kb.

Figure 4 shows that the *Bcl* I stoichiometric RFLP differences between *cam* and *jun* cpDNAs were truly characteristic of the cytoplasm whereas the sub-stochio-

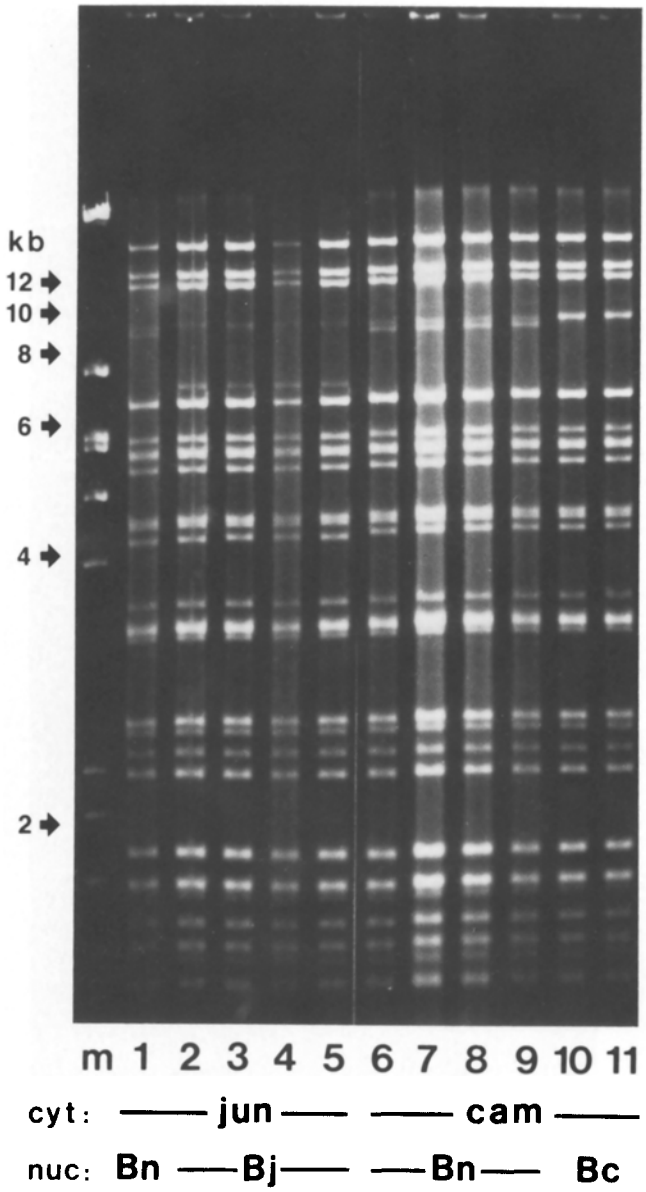

Fig. 4. *Bcl* I cpDNA restriction fragment patterns of *jun* and *cam* cytoplasms in different nuclear backgrounds. 1% agarose gel. Lane 1: *jun* in *B. napus* cv. 'Regent'; lane 2: *jun* in *B. juncea* cv. 'ZEM'; lane 3: *jun* in *B. juncea* cv. 'Bursa Bold'; lane 4: *jun* in *B. juncea* line LG1; lane 5: *jun* in *B. juncea* cv. 'Domo'; lane 6: *cam* in *B. napus* cv. 'Bronowski'; lane 7: *cam* in *B. napus* cv. 'Rabo'; lane 8: *cam* in *B. napus* cv. 'Hanna'; lane 9: *cam* in *B. napus* cv. 'Liné; lane 10: *cam* in *B. campestris* cv. 'Tobin' and lane 11: *cam* in a Swedish *B. campestris* line. Lane m as in Fig. 1

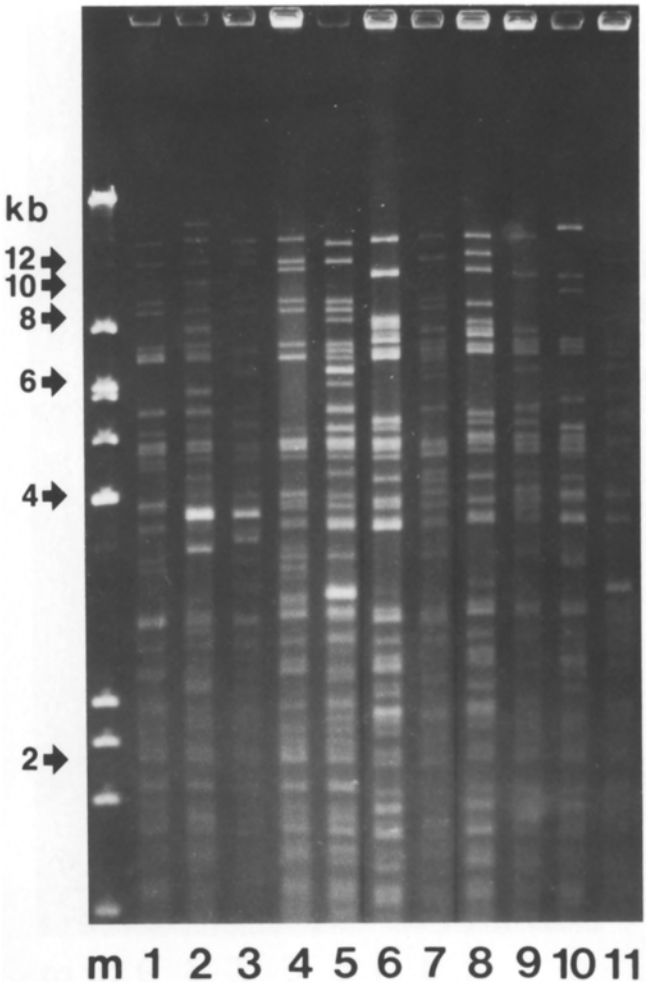

Fig. 5. *Eco* RI mtDNA restriction fragment patterns of 'wild' *Brassica* species on 1% agarose gel. Lane 1: *B. alboglabra;* lane 2: *B. amplexicaulis;* lane 3: *B. barrelieri;* lane 4: *B. certa;* lane 5: *B. deflexa;* lane 6: *B. fruticulosa;* lane 7: *B. gravinae;* lane 8: *B. maurorum;* lane 9: *B. rupestris;* lane 10: *B. spinescens* and lane 11: *B. tournefortii.* Lane m as in Fig. 1

metric differences varied with the nuclear component of the cells. All *jun* cpDNAs (lanes 1 to 5) exhibited the 4.2 kb fragment and all *cam* cpDNAs (lanes 6 to 11) had the 4.3 kb fragment regardless of the nuclear genome with which they were combined. However, *jun* cpDNA in combination with its natural *B. juncea* nucleus exhibited sub-stoichiometric fragments of 9.6 and 7.0 kb (lanes 2 to 5), but when in combination with a *B. napus* nucleus, sub-stoichiometric fragments of 9.3 and 9.0 kb were observed (lane 1). Similarly, *cam* cpDNA in combination with its natural *B. campestris* nucleus possessed a sub-stoichiometric fragment of 9.6 kb (lanes 10 and 11) but when in combination with a *B. napus* nucleus this fragment was also replaced with fragments of 9.3 and 9.0 kb (lanes 6 to 9). Since the sub-stoichiometric cpDNA *Bcl* I fragments exhibited by *jun* cytoplasm in a *B. napus* cell are identical to those of *cam* and *nap* cytoplasms in the same nuclear background, nuclear control over chloroplast genome organization is clearly evident. Reciprocal backcrossing programs have recently indicated that a similar nuclear control is exerted over maize mtDNA (Escote 1986).

An indication of the ubiquity and reliability of this assay is that all 112 of the various nucleo-cytoplasmic

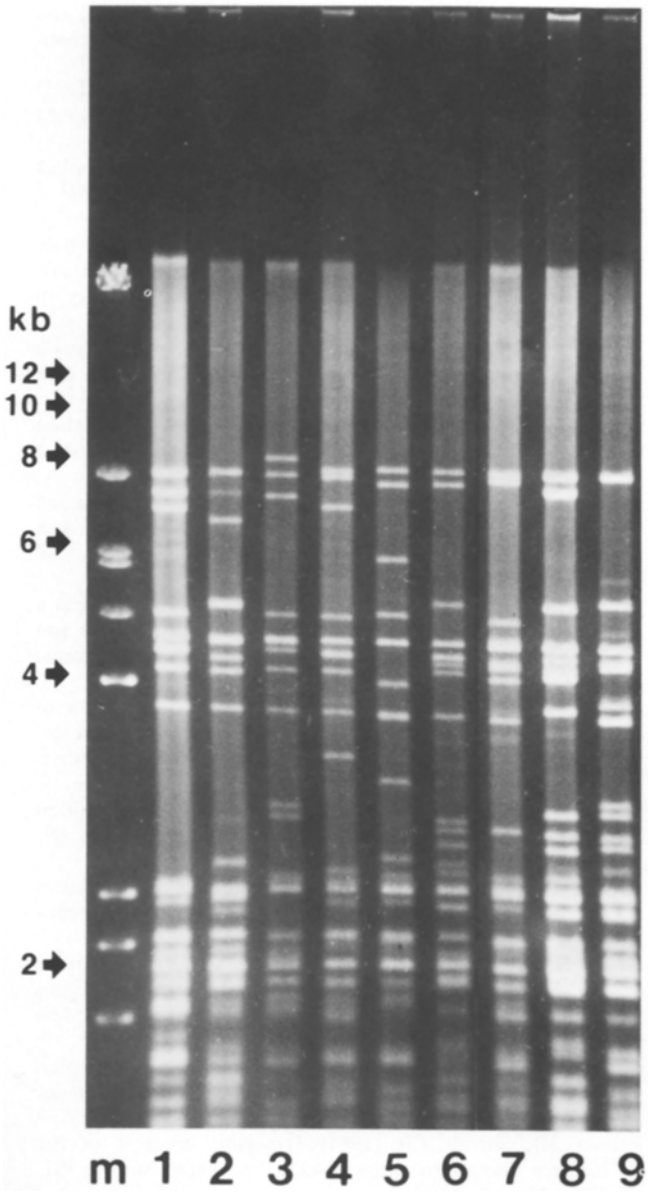

Fig. 6. *Eco* RI cpDNA restriction fragment patterns of 'wild' *Brassica* species on 1% agarose gel. Lane 1: *B. alboglabra;* lane 2: *B. amplexicaulis;* lane 3: *B. barrelieri;* lane 4: *B. certa;* lane 5: *B. elongate;* lane 6: *B. fruticulosa;* lane 7: *B. rupestris;* lane 8: *B. spinescens;* lane 9: *B. tournefortii.* Lane m as in Fig. 1

combinations detailed in Table 1 and all 26 somatic cybrids (Barsby et al. 1987) produced one of the characteristic cpDNA and mtDNA restriction patterns shown in Figs. 1, 2 and 3. In addition, characteristic mtDNA (Fig. 5) and cpDNA (Fig. 6) *Eco* RI restriction patterns were obtained from 13 'wild' *Brassica* species.

Discussion

Restriction enzyme fragmentation of *Brassica* mtDNAs has previously been employed to determine restriction patterns and physical maps of *B. napus, B. campestris* and *B. oleracea* genomes (Chetrit et al. 1984; Erickson et al. 1986a; Lebacq and Vedel 1981; Palmer and Shields 1984), identify DNA recombination in hybrids (Chetrit et al. 1985) and determine the structure of the 11.3 kb plasmid (Erickson et al. 1986b; Palmer et al. 1983a). Restriction enzyme fragmentation of *Brassica* cpDNAs has been used to obtain restriction patterns and a physical map of *B. napus* (Erickson et al. 1986a; Lebacq and Vedel 1981; Vedel and Mathieu 1983), determine the inheritance of the organelle in hybrids (Pelletier et al. 1983) and clarify the evolutionary origin of *Brassica* cytoplasms (Erickson et al. 1983; Palmer et al. 1983b). Methods detailed in these reports were unsuitable as rapid single plant cytoplasmic DNA screening procedures because most required large amounts of leaf tissue and, consequently, destroyed the donor plants. In addition, no single restriction enzyme was identified which could differentiate among the mitochondrial and chloroplast genomes of interest. The cytoplasmic DNA characterization assay described here was developed to overcome this deficiency.

The advantages and uses of the rapid, non-destructive, single leaf RFLP mtDNA and cpDNA assay system reported here are:

1. It allows the appropriate restorer and maintainer lines for any *cms* plant to be immediately identified. To achieve such a goal by traditional plant breeding techniques requires several years of backcrossing to a large number of maintainer and restorer lines. This, of course, is very labor and space intensive and therefore extremely expensive. The RFLP assay can be completed in less than three days at minimal expense. One technician can perform several assays concurrently.

2. It allows the definitive identification of somatic hybrids and cybrids produced by protoplast fusion experiments. We have used the assay to detect novel mitochondria and chloroplast combinations in *B. napus* cybrids (Barsby et al. 1987).

3. It allows genetic variation, at the cytoplasmic DNA level, to be detected in natural plant populations and in plants regenerated from tissue culture where such somaclonal variation is very common. Plant breeders are anxious to develop crops with non-uniform cytoplasmic genomes as an assurance against epidemics similar to the *Helminthosporium maydis* race T disaster that surprised the hybrid maize industry in 1970 (Ullstrup 1972).

4. The degree of seed contamination in any seed batch can be determined provided that contaminating seed possesses a different cytoplasm. Unfortunately, seed mixes are often encountered in small seed crops such as canola because the cleaning methods for harvesting equipment are not totally effective. Obviously, mixed seed lots cannot be tolerated in, for example, commercial hybrid seed production where the female parent line has to be completely *cms.*

5. The success of organelle transformation experiments can be monitored. The recent demonstration of chloroplast transformation by 'foreign' DNA (De Block et al. 1985) will undoubtedly make this a major research emphasis for many plant molecular biologists.

An additional benefit of the assay system is that most of the *Brassica* mtDNA and cpDNAs can be differentiated by a single enzyme, *Eco* RI. Not only does this facilitate the preparation of the restriction enzyme reactions, eg., only one buffer stock is required, but *Eco* RI is the cheapest and one of the most stable commercially available restriction enzymes.

The rapidity of the assay can be further increased by extracting mtDNA and cpDNA from the same homogenate. Best results were obtained when the homogenization and filtration steps were those described for mtDNA extractions. After the initial centrifugation step of 1,000 × g for 10 min, the supernatant was further processed for mtDNA extraction exactly as described. However, instead of discarding the pellet it was resuspended in 10 ml Buffer E and then processed as outlined for cpDNA isolation. cpDNA isolated in this manner was restrictable and only marginally inferior (higher background fluorescence on agarose gels) to that extracted completely separately from mtDNA.

The power of the asay is such that it has been able to (1) differentiate cpDNA of Shiga's nap *cms* system from male fertile nap cpDNAs (Fig. 2) suggesting that each cytoplasm has evolved from a different origin, or that the two cytoplasms diverged from a common parent at an early point in evolution (2) identify two different types of mtDNA organization in our *R. sativus* accessions (data not shown), (3) differentiate between mtDNAs and cpDNAs of several 'wild' or uncultivated *Brassica* species which represent potential new sources of *cms*, (4) demonstrate that chloroplast genome organization is under nuclear control (Figs. 3 and 4) and (5) assist our understanding of cytoplasmic genetics in *Brassica* which, unfortunately, is not well developed.

Although the 'wild' *Brassica* species represented a wide array of different plant morphology, eg., leaf size, cuticle thickness and hairyness, the mt and cpDNA extraction procedures produced DNA of sufficient quality for restriction enzyme analyses. This finding, together with the successful cytoplasmic DNA extractions from the more common species detailed in this study, suggests that these procedures should be applicable to most plant species.

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