

Chloroplast DNA of Brassica

L. Erickson, I. Grant, B. Cardy, W. Beversdorf, Crop Science Dept.,
University of Guelph, Guelph, Ontario Canada, N1G 2W1

Introduction:

Restriction patterns of chloroplast (ct) and mitochondrial (mt) DNA have contributed substantially in recent years to our understanding of organelle genomes in higher plants. Such patterns have been used in the following areas of research:

1. To determine cytoplasmic variability within a crop or species (Levings and Pring 1977);
2. To investigate cytoplasmic mutants such as male sterility (Pring et al. 1979);
3. To determine taxonomic relationships between species (Vedel et al. 1981; Kung et al. 1982);
4. To detect recombination within and between organelle genomes following fusion experiments (Bélliard et al. 1979).

We present here data from preliminary studies of ct DNA in Brassica. These studies fall into the first three categories of research mentioned above and will be presented in the same order.

Cytoplasmic Variation in Rapeseed:

The demonstration of the cytoplasmic inheritance of disease susceptibility in maize (Ulstrup 1972) and tobacco (Durbin and Uchytel 1977) illustrates the need to search for and maintain cytoplasmic diversity in crop species. Our survey of rapeseed cytoplasms involved 12 B. napus cultivars or accessions from seven countries: Canada (Tower, ATR-5Tw), China (CR-1), France (Jet Neuf, Raphanobrassica), Japan (Asahe-natane, Isuzu-natane, CMS(E)-Shiga), Poland (Tanpol, Bronowski), Russia (Mitnickij), Sweden (Brink); and five B. campestris cultivars or accessions from three countries: Canada (CZR6-914, Candle, Torch), Russia (Latvijskaja) and Sweden (SV761300).

A non-rapeseed cultivar from each species was included for comparison: a rutabaga in B. napus (cv. York, Canada) and Bok choy (cv. unsp., Japan) in B. campestris.

The ct restriction patterns of three B. napus cultivars (ATR-5Tw, Bronowski and Isuzu-natane) appear to be identical to those in B. campestris for all 15 enzymes. ATR-5Tw was derived by backcrossing the B. napus cultivar Tower onto a wild triazine resistant biotype of B. campestris as female (Beversdorf et al.

1980) and the consistent campestris restriction patterns of this cultivar demonstrate the maternal inheritance of the ct genome in Brassica. Certain seed quality characteristics of Bronowski suggest that it also originated from such an interspecific cross (K. Downey, personal communication), a process in which it could have acquired a campestris cytoplasm. The history of rapeseed breeding in Japan points to a similar explanation for the campestris patterns of Isuzu-natane. When B. napus was introduced to that country at the end of the last century, it was extensively hybridized with already well-established cultivars of B. campestris which is why more than 60% of the registered Japanese rapeseed cultivars are from interspecific crosses (Namai et al. 1980).

In contrast to the above three cultivars, however, the other nine rapeseed cultivars in B. napus, as well as rutabaga, gave campestris restriction patterns with some enzymes, oleracea patterns with others and patterns distinct from both oleracea and campestris with still other enzymes. Significantly, each of these nine cultivars had almost identical patterns with each enzyme; that is, all resembled the campestris pattern, the oleracea pattern, both patterns or neither depending on the enzyme. This is evidence of a separate napus ct genome distinct from that of B. oleracea and B. campestris but sharing homologies with both. The existence of uniformly campestris patterns in the ct DNA of napus cultivars such as Bronowski and Isuzu-natane probably results from their origin in B. campestris X B. napus crosses long after B. napus had evolved as a separate species. Other examples of cytoplasmic introgression by campestris into napus will undoubtedly be found in the future as this cross is fairly easy to perform in either direction. As yet no napus ct DNA patterns have been found in campestris cultivars.

As reported by Vedel et al. (1982), the B. napus male sterile cytoplasm derived from radish (Raphanobrassica) displayed ct patterns characteristic of Raphanus and usually quite distinct from Brassica.

Within the ct genome of B. napus itself, as represented by the above-mentioned nine cultivars, there appears to be very little polymorphism at the nucleotide level. The large Bcl I fragment #1 is missing in Brink due apparently to a mutation giving rise to a restriction site in that fragment. Such a mutation could originate from a variety of alterations ranging from a base pair substitution to an inversion.

Within the ct genome of B. campestris, as well, there seems to be very little variation. Candle appears to have an extra fragment in digests with Hind III, Bam HI and Eco RI. This extra fragment is probably due to an alteration in restriction sites and not to a

difference in genome size since in several other digests Candle has patterns identical to other campestris cultivars. However, small differences in fragment size may not be detected in comparisons of restriction patterns, especially when large fragments are involved (Gordon et al. 1982).

Cytoplasmic Male Sterility in Rapeseed

According to Shiga et al (1980) most napus cultivars from Europe and Japan have an S (sterility-inducing) or "nap" cytoplasm. The Japanese cultivars with N (non-sterile-inducing) cytoplasm were apparently derived from two interspecific (B. campestris X B. napus) crosses. Shiga has designated the N cytoplasm as the "cam" cytoplasm and suggests that it originated from B. campestris. As might be expected, nap-type cytoplasm also occur in campestris (ibid, 1980). Nap cytoplasm have probably been introduced to B. campestris by hybridization; however, it may be that differences exist within the campestris cytoplasm itself, differences which have so far escaped detection by restriction digests, and that some of these different cytoplasm in association with the rf genes of Bronowski or Isuzu-natane give rise to male sterility.

Our results to date support the contention that the N cytoplasm of Bronowski and Isuzu-natane has been derived from B. campestris. Ct DNA restriction patterns of these two cultivars are identical and unmistakably of the campestris type. As well, ct DNA from Shiga's CMS(E) is of the napus type. It appears, then, that certain nuclear (rf) genes, which have originated in association with certain campestris cytoplasm (Bronowski, Isuzu-natane for eg.), cause male sterility when associated with napus cytoplasm.

Recently Palmer et al (1983) provided evidence for a "plasmid"-like DNA in the mitochondria of campestris cultivars and in Bronowski and suggested a possible role for such a plasmid in cytoplasmic male sterile systems in rapeseed. We are currently investigating the occurrence of such a plasmid.

Phylogeny of Chloroplast Genomes in Brassica

Lebacqz and Vedel (1981) reported that Sal I digests of ct and mt DNA from B. oleracea, napus, campestris, juncea, nigra and carinata generally supported the taxonomic classification within Brassica but did not reveal the cytoplasmic origin of the three amphiploid species (B. napus, juncea and carinata).

On the basis of electrophoretic patterns of ct DNA digested with 15 restriction enzymes (Table 1) we have divided the ct genomes of Brassica into two groups: complex one (B. oleracea, napus, campestris and juncea and complex two (B. nigra and carinata) (Erickson et al. in press).

Table 1. Classification of electrophoretic patterns of chloroplast DNA extracted from six Brassica species and digested with 15 restriction endonucleases.

Enzyme	Restriction patterns and species of <u>Brassica</u>					
	<u>oleracea</u>	<u>napus</u>	<u>campestris</u>	<u>juncea</u>	<u>nigra</u>	<u>carinata</u>
	n=9	n=19	n=10	n=18	n=8	n=17
Sph I	1*	1	1	1	1	1
Sst I	1	1	1	1	1	1
Sal I	1	1	1	1	2	2
Xho I	1	1	1	1	2	2
Sma I	1	1	1	1	2	2
Kpn I	1	1	1	1	2	2
Pvu II	1	1	1	1	2	2
Xba I	1	1	2	2	3	3
Eco RI	1	1	2	2	3	4
Bam HI	1	1	2	2	3	3
Hind III	1	1	2	2	3	3
Pst I	1	2	2	2	3	3
Bgl II	1	2	2	2	3	3
Cla I	1	2	1	1	3	3
Bcl I	1	2	1	1	3	3

* For each enzyme a pattern is given a number (starting arbitrarily with one at B. oleracea and moving counter-clockwise around the triangle of U) and species which share the same pattern have the same number for this enzyme.

Classifications based on morphology (Schulz 1919), chromosome pairing in hybrids (Mizushima 1950) and seed proteins (Vaughan et al 1966) also support a bipartite division of the ct genome in Brassica.

Of most significance, however, are the comparisons of the restriction patterns of the amphiploids with those of their putative parents as indicated in the triangle of U (1935). With two minor exceptions the patterns of B. carinata and juncea were the same as those of B. nigra and campestris, respectively. In the case of one exceptional digest (Eco RI) the difference between carinata and nigra was very slight compared to that between carinata and oleracea and similarly in the other exceptional case (Bgl II) there is only a minor difference between juncea and campestris. This indicates that the ct genomes of B. carinata and B. juncea are derived from those of B. nigra and B. campestris respectively and that the ct genomes of these two amphiploids have evolved little since their time of origin.

Evidence for a distinct napus ct genome was given above in the section on cytoplasmic variability in rapeseed. The similarities

and differences between the 15 restriction patterns of napus (N) and those of campestris (C) and oleracea (O) can be represented as follows:

Set 1 : O = N = C (seven enzymes)

Set 2 : O = N ≠ C (four enzymes)

Set 3 : O ≠ N = C (two enzymes)

Set 4 : O = C ≠ N (two enzymes)

The extensive homology between all three species is evident not only from the identical patterns of set one, but also from those of other sets where the differences between species involve only one or two bands. The greater similarity between napus and oleracea (set two) than between napus and campestris (set three) suggests that the ct genome of napus derived from that of oleracea. The differences between napus and campestris (set two) would thus be due to divergence between oleracea and campestris in the pre-napus period and to alterations in campestris during the napus period. Such an hypothesis would explain the differences of set three most simply as a result of alterations in the oleracea genome during the napus period and those of set four as a result of alterations in the napus genome in the same period.

Investigation into rapeseed and related cytoplasms will no doubt accelerate in the coming years and, in conjunction with rapidly developing techniques of molecular and cell biology, perhaps so will our ability to alter these cytoplasms in agronomically useful ways.

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