

Chloroplast and mitochondrial DNA variations between different cms lines of rapeseed.

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Introduction

Cytoplasmic male sterility (cms) is a maternally inherited trait found in a wide range of plant species. cms has arisen in intraspecific as well as interspecific crosses, spontaneously or after mutagenic treatment. Alloplasmic cms has led to the concept that cms results from incompatibility between the nuclear genome of one species and the cytoplasm of another. Substantial evidence suggests that the mitochondrial (mt) genome rather than the chloroplast (ct) genome is implicated in the cms trait. Comparisons of organelle DNA from normal and cms lines by means of restriction patterns have provided information in corn, wheat, tobacco, faba beans, sugarbeet and sorghum (Leaver and Gray 1982, Levings 1983, Pring and Lonsdale 1985). In rapeseed, characterization of cms lines at the molecular level has focussed largely on the *Raphanus* system. This cytoplasm derived from *Raphanus sativus* Ogura type (Bannerot et al., 1974), differs from that of normal, fertile *B. napus* with respect to mt DNA restriction patterns and mt *in vitro* polypeptide synthesis (Vedel et al. 1982), physical and gene mapping of chloroplast DNA (Vedel and Mathieu 1983) and thylakoid proteins (Rémy and Ambard-Bretteville 1983). Cytoplasmic hybrids regenerated from protoplast fusion experiments demonstrate that this cms is not associated with the chloroplast (Pelletier et al., 1983) and that recombination of mt genomes occurs in Brassica (Chétrit et al. 1985, Vedel et al. 1986).

In this study, we report a comparison, on the basis of restriction patterns, of ct and mt DNAs from different cms lines in rapeseed.

Materials and Methods

Male sterile *B. napus* plants with *Diploetaris muralis* cytoplasm were obtained by backcrossing the cms *B. campestris* line obtained by Hinata and Konno (1979) with *B. napus* cv. Mangun (Pellan-Delourme, 1986; Pellan-Delourme and Renard 1987). A rapeseed line, with the *B. juncea* cytoplasm (Rawat and Anand, 1979) was provided by Dr P.H. Williams (Department of Plant Pathology, Univ. Wisconsin, Madison, USA). This line was then backcrossed with *B. napus* cv. Bienvenu. cms rapeseed plants with the *B. nigra*

cytoplasm (Pearson, 1972) were obtained as indicated by Pellan-Delourme et al. (these proceedings). Seeds of male sterile plants with Polima cytoplasm (Fu, 1981) were kindly supplied by Dr I. Bartkowiak-Broda (Plant Breeding and Acclimatization Institute, Poznan, Poland). For purposes of analysing organelle DNA, all these plants were grown in the field and seeds were obtained from open pollinations. In addition seeds were taken on the male fertile *B.napus* cultivars "Brutor" and "Bronowski".

Isolation and analysis of ct and mt DNAs were made as previously described (Vedel et al., 1982 ; Pelletier et al., 1983).

Results and Discussion

MITOCHONDRIAL GENOMES

Among higher plants so far analysed, Brassica species possess the simplest mt genomes (Lebacqz and Vedel 1981). In Fig.1, Sal I mt DNA restriction patterns illustrate the alloplasmic nature of cms systems in *B.napus* rapeseed. Male

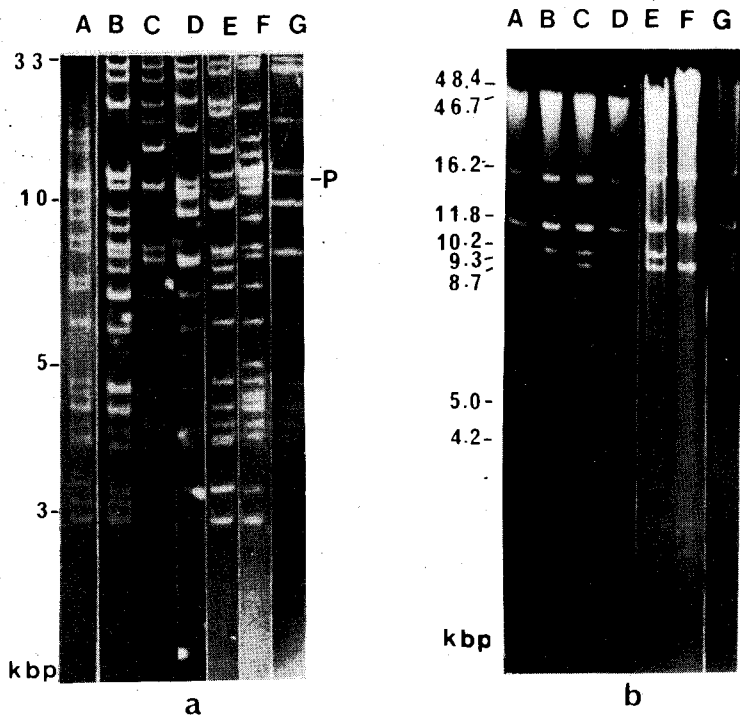


Fig.1 : Sal I restriction patterns of mt DNA (a) and ct DNA (b) from cms *B.napus* rapeseed lines with *B.juncea* (A) *B.nigra* (B), *Diplotaxis* (C), *Polima* (D), and *Ogura* (F) cytoplasms, and from fertile *B.napus* rapeseed lines with *Brutor* (E) and *Bronowski* (G) cytoplasms. The restriction fragments were separated by electrophoresis on 0.7% agarose gels. P locates the 11.3 kb mitochondrial plasmid. The scale of molecular weights is in kbp or 10^3 base pairs.

sterile *B.napus* rapeseed line with Ogura cytoplasm was already described by Pelletier *et al.* (1983). The *Sal* I restriction pattern of Bronowski mt DNA is identical to that previously obtained with *B.campestris* mt DNA (Lebacqz and Vedel 1981; Vedel *et al.* 1986; Eriksson *et al.*, 1986). The 11.3 kb mitochondrial plasmid which is not cleaved by the *Sal* I enzyme (Palmer *et al.* 1983) is present in the seven cytoplasms, it only appears as a faint band in lanes corresponding to Diplotaxis, Brutor and Bronowski cytoplasm. As previously mentioned (Chétrit *et al.* 1985; Kemble *et al.* 1986) no close association exists between the presence of the plasmid and cms.

CHLOROPLAST GENOMES

ct genomes are highly conserved and less complex than the corresponding mt genomes as illustrated in Fig.1b. *B.juncea*, *B.nigra* and Ogura cytoplasms are characterized by specific ct DNA *Sal* I diagrams, whereas Diplotaxis, Polima, Brutor and Bronowski ct DNAs cannot be distinguished. *Cla* I

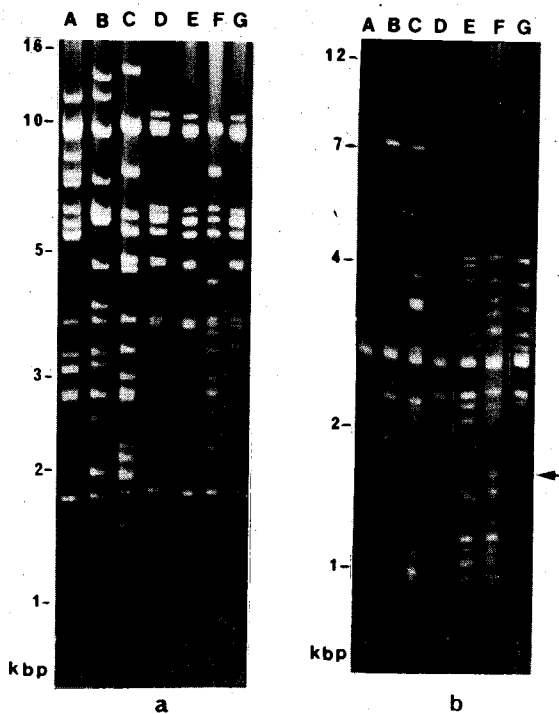


Fig.2 : *Cla* I (a) and *Hpa* II (b) restriction patterns of ct DNA from cms *B.napus* rapeseed lines with *B.juncea* (A), *B.nigra* (B), Diplotaxis (C), Polima (D) and Ogura (F) cytoplasms, and from fertile *B.napus* rapeseed lines with Brutor (E) and Bronowski (G) cytoplasms. The restriction fragments were separated by electrophoresis on 0.7% agarose gels. Difference between Polima and Bronowski ct DNAs is located with an arrow in *Hpa* II diagrams.

enzyme led to specific patterns with *B. juncea*, *B. nigra*, *Diplotaxis*, *Brutor* and *Ogura* ct DNAs. In contrast, *Polima* and *Bronowski* give identical ct DNA patterns (Fig. 2a). Each ct DNA can be distinguished on the basis of *HpaII* ct DNA restriction patterns (Fig. 2b). *Sal I* and *Hpa II* patterns of *Bronowski* ct DNA are identical to that previously found with *B. campestris* ct DNA (Vedel and Lebacqz 1981; Pelletier et al. 1983). On the other hand, the origin of the *Polima* cytoplasm is unknown and these results indicate that *Polima* mt and ct DNAs are most similar to that of *B. campestris*, as suggested previously by Erickson et al. (1986).

In summary, restriction patterns of mt and ct DNAs in rapeseed reveal the alloplasmic nature of cms in this crop. These patterns represent valuable markers for following the fate of organelles and their DNAs in fusion and transformation experiments.

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