MACROMOLECULAR ANALYSIS OF CYTOPLASMIC POLYMORPHISM AND CHARACTERIZATION OF CYTOPLASMIC PARASEXUAL HYBRIDS OF BRASSICA NAPUS

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The rape Brassica napus is a natural amphidiploid (2 n = 38) combining the chromosomal basic sets of B. campestris (2 n = 20) and B. oleracea (2 n = 18). Cytoplasmic male sterility of B. napus arose from intergeneric crosses involving a cytoplasmic male sterile (cms) japanese line of Raphanus sativus (1, 2). The cms lines of B. napus present two maternally inherited traits, cytoplasmic male sterility and chlorophyll deficiency both resulting from interactions between the nuclear genetic system of B. napus and organellar genetic ones of radish. Unfortunately, chlorophyll deficiency prevents the agronomical use of cms B. napus. Normal and cms lines of B. napus have been distinguished previously by restriction analysis of chloroplast (cp) and mitochondrial (mt) DNAs, and in vitro protein synthesis products of isolated mitochondria (3).

In this study, we present : (a) physical maps for each type of B. napus cp DNA using the restriction enzymes Sal I, Sma I, Bgl I and Kpn I. In addition, the positions of rRNA and of two protein genes have been localized on the physical maps of these DNAs; (b) two dimensional gel mapping of thylakoid proteins from N and cms lines of B. napus; (c) the characterization of cms parasexual hybrids corrected for chlorophyll deficiency,by analysis of the chloroplast macromolecules.

MATERIALS AND METHODS

Plant materials : N and cms lines of B. napus were grown in a greenhouse (2). Rapeseed cytoplasmic somatic hybrids (cybrids) were regenerated after protoplast fusion as described previously (4, 5).

Physical and gene mapping of cp DNA : Chloroplasts were prepared from three-weeks old leaves and cp DNA isolated from either DNase-treated or untreated chloroplast pellets by using CsCl-ethidium bromide gradients. Physical maps were constructed according to the restriction multienzyme method using a semi-preparative electrophoretic isolation of the restriction fragments on low-gelling temperature agarose gels (6). Gene mapping was made using the molecular hybridization procedure of Southern (7).

Two dimensional separation of thylakoid polypeptides. The techniques of 2 D - separation as well as the isolation of thylakoid membranes from Brassicae have been described elsewhere (8). The identification of the α and β subunits of the chloroplast ATPase was performed by comparison with the respective position of these subunits in 2 D - separations realized with purified coupling factor (9).

Biochemical characterization of the cybrids. A simple and rapid procedure was used to analyse cp DNA from no more than 1 to 3 g of fresh leaves (4). The different cytoplasms were distinguished both with the Bgl I restriction patterns of their cp DNAs and the 2 D - patterns of their thylakoid polypeptides.

RESULTS and DISCUSSION

Physical and gene mapping of N and cms rape cp DNAs. Using the restriction endonucleases Sal I, Sma I, Bgl I and Kpn I, physical maps were constructed and compared (Figure 1). The overall structural organization of N and cms B. napus appears to be rather similar to that of cp DNAs of other higher plants, the leguminosae excepted (10). It is composed of two identical sequences (each about 15 Md) arranged as an inverted repeat separated by two single copy-regions of different sizes (about 54 and 15 Md). In both genomes, the ribosomal RNAs are encoded by duplicate genes situated at one end of the inverted repeat. The two cp genomes are distinguished by a point mutation in the rRNA locus (Sma 9 site). Genes for the large subunit (LS) of ribulose-1,5-biphosphate carboxylase and a 32 kd photosystem II polypeptide (P II) are separated by a minimum of 30 Md of DNA within the large single copy region. Probes used are distinct restriction fragments of spinach cp DNA which contain the LS and the P II genes (11). The genes for LS and P II are in similar locations in N and cms genomes as in the spinach cp genome.

2 - D electrophoresis of cp proteins - As seen on figure 2, although there was extensive homology between protein maps of thylakoids isolated from the two lines, these could be distinguished by the spots corresponding to the β subunits of the coupling factor CF1 from the ATPase complex.

We have observed that restriction patterns of cp and mt DNAs and 2 - D patterns of thylakoid proteins from cms rape were identical to the corresponding patterns from the cms japanese radish used to transfer the cms trait into the rape, indicating that the cytoplasm of the cms rape was inherited maternally.

The biochemical characterization of cybrids was made using Bgl I restriction patterns (Figure 3) of cp DNA and 2 - D patterns of thylakoid proteins. Cp DNAs of N. and cms B. napus were distinguished by their Bgl I patterns with two specific bands each. The N B. napus Bgl I pattern was identical to the corresponding one in B. campestris

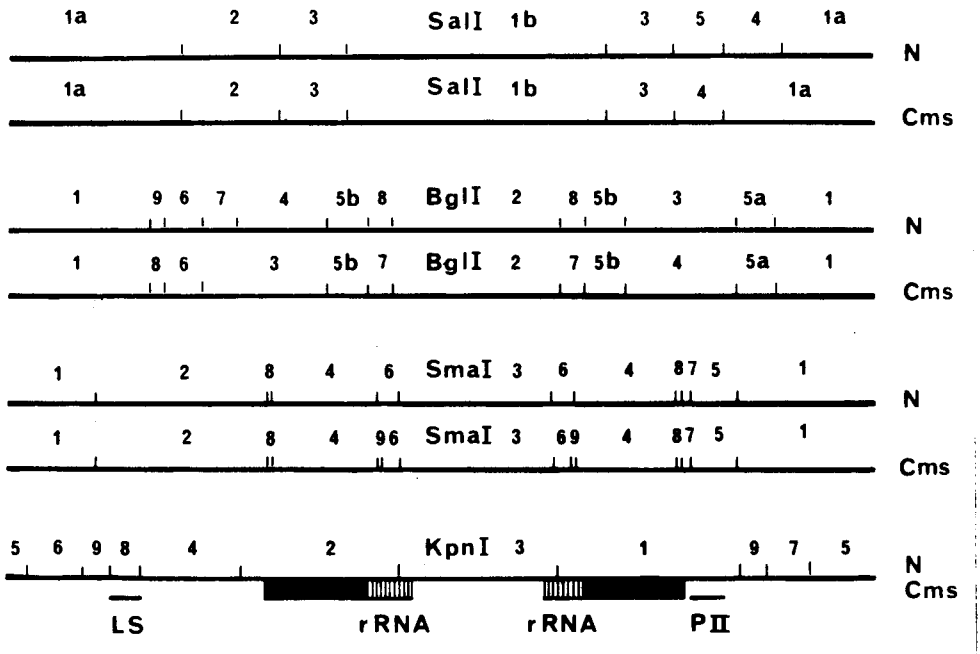

Fig.1 - Comparison of the physical maps of cp DNAs from N and cms rapes. The circular maps were linearized by a cut in the large single copy region. The thick lines represent the two copies of the inverted repeat region (molecular weights of restriction fragments are given in ref. 6).

(figure 3). Hpa II patterns of cp DNAs allowed us to differentiate between B. napus and B. campestris (figure 4). This is not the case for all B. napus cultivars : cv "Bronowski" has shown a pattern identical to B. campestris. This fact may suggest a different phylogenetic origin for this cultivar, although other hypotheses cannot be ruled out.

As described elsewhere (4,5) five regenerated plants have been found to be cms with the chloroplasts of B. napus and one regenerated plant to be cms with the chloroplasts of atrazine-resistant B. campestris.

Preliminary analysis of mt DNA indicates that cybrids possess specific mt DNAs, distinct from parental ones, as was described previously with tobacco cybrids (12). The plasmid-like molecule found in mitochondria of several Brassica species (13) has been evidenced in the rapeseed cybrids.

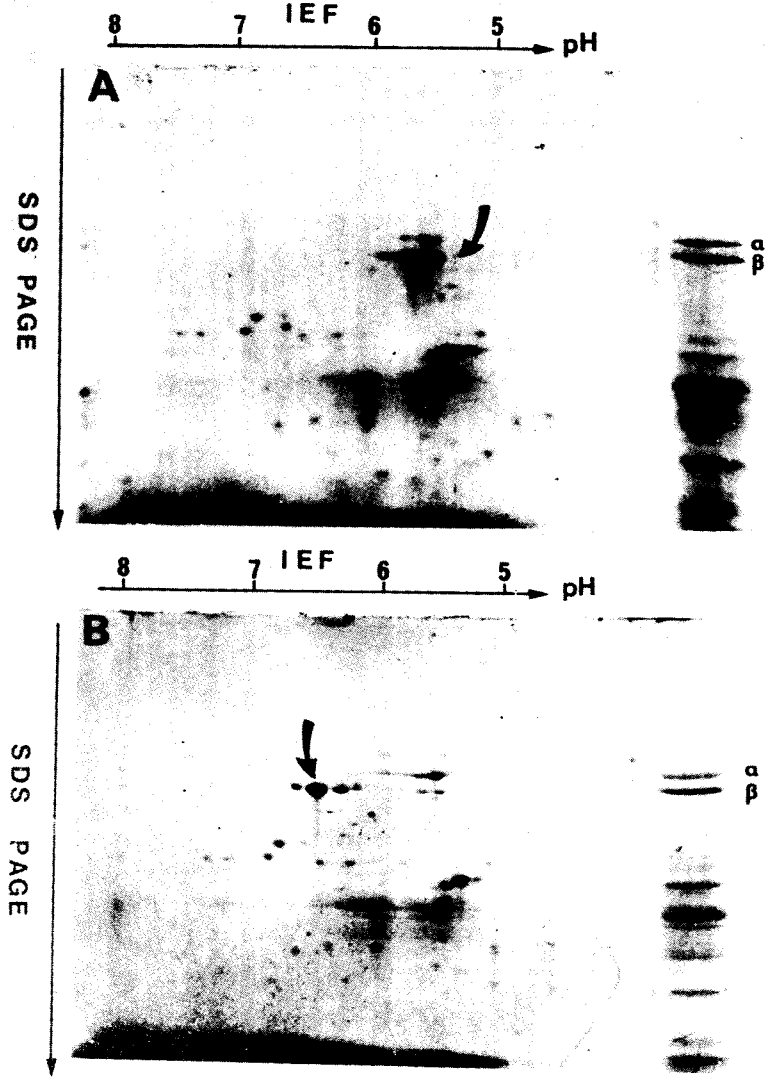

Fig. 2 - Thylakoid polypeptides separated on 2 D - gel electrophoresis and stained with Coomassie blue. A : normal B. napus; B : cms B. napus. The big arrows indicate the position of β subunits of coupling factor. On the right side of the figure are one dimensional SDS-PAGE of thylakoid polypeptides.

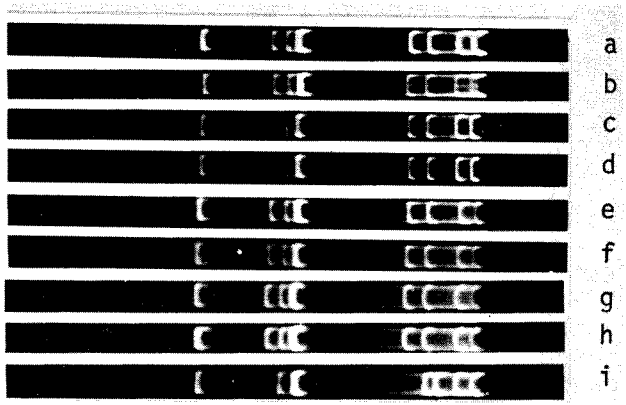

Fig. 3 - Bgl I restriction patterns of cp DNAs from (a) N B.napus, (b) cybrid 27, (c) plant 41, (d) cybrid 58, (e) cybrid 118, (f) cybrid 23, (g) cybrid 77, (h) triazine resistant B. campestris,(i) cms B. napus (radish cytoplasm). The restriction fragments were separated by electrophoresis on 20 cm vertical slab gels containing 0.7 % agarose.

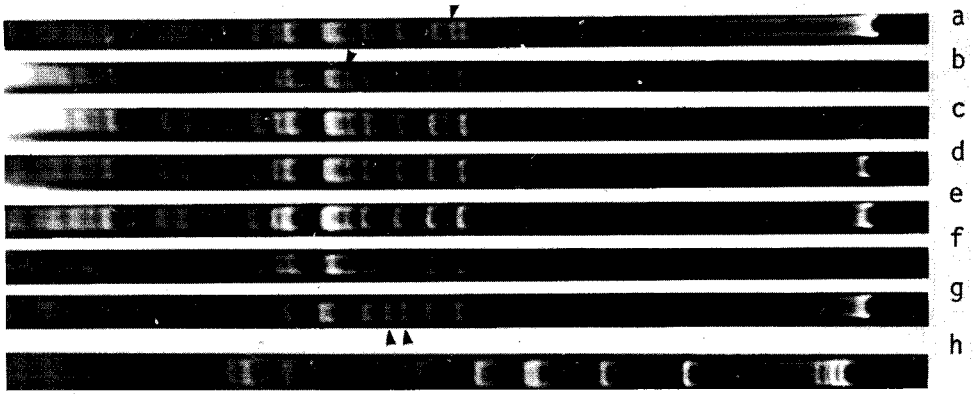

Fig. 4 - Hpa II restriction patterns of cp DNAs (a) N B. napus, (b) cybrid 77 , (c) B. napus with B. campestris atrazine resistant cytoplasm, (d) atrazine resistant B. campestris, (e) atrazine sensible B. campestris, (f) B. napus cv. Bronowski,(g) cms B. napus (radish cytoplasm), (h) molecular weight standard (Marker II + Marker III Boehinger-Mannheim). The restriction fragments were separated by electrophoresis on 40 cm vertical slab gels containing 0.7 % agarose.

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