MOLECULAR, PHENOTYPIC AND GENETIC CHARACTERIZATION OF MITOCHONDRIAL RECOMBINANTS IN RAPESEED.

PELLETIER G., PRIMARD C.- Laboratoire de Biologie Cellulaire. CNRA -78000 VERSAILLES - FRANCE
VEDEL F., CHETRIT P. - Laboratoire de Génétique Moléculaire des Plantes - Bât.360 - 91405 ORSAY - FRANCE
RENARD M., PELLAN-DELOURME R., MESQUIDA J. - Station d'Amélioration des plantes et Laboratoire de Zoologie - INRA - 35650 LE RHEU - FRANCE

I. INTRODUCTION

Several systems of cytoplasmic male sterility are known in rapeseed, although none is used in practise because of difficulties encountered, either with maintainer or restorer genes, or morphological and physiological defects. Among them, at least four are known to result from alloplasmic situations. The nucleus of *B. napus* was introduced into the cytoplasm of *Brassica nigra*, *Brassica juncea*, *Diplotaxis muralis* and *Raphanus sativus* of Ogura type (Ogura 1968). The latter is of interest because it results in a complete male sterility and restorer genes have been introduced in rapeseed from radish (Heyn, 1978).Nevertheless *Brassica* plants bearing radish cytoplasm exhibit yellowing especially at low temperature and maintain low chlorophyll level at higher temperature (Bannerot *et al.*, 1977, Rousselle 1981, Mc Collum 1981). Moreover flowers of these plants have less developed nectaries and reduced nectar production. This defect is important in hybrid seed production, since the cms plants are less attractive for bees, the most important vectors in cross pollination (Renard and Mesquida, 1983).

Another type of alloplasmic situation is of interest for *Brassica* crops. An atrazine-resistant *B. campestris* biotype has been discovered (Maltais and Bouchard 1978). This resistance has been shown to be maternally inherited and associated with the reducing side of photosystem II (psb A gene). Indeed alloplasmic *B. napus* lines with *B. campestris* atrazine-resistant cytoplasm have been shown to be fully resistant to this herbicide (Beversdorf *et al.*, 1980. Renard et al in these Proc.). Atrazine-resistance could be useful as a weed control strategy.

Protoplast fusion offered the possibility to improve the phenotypical characteristics of Ogura type cytoplasmic male sterility and to combine triazines resistance and male sterility in the same cytoplasm. This allows the commercial production of F_1 hybrid

olants resistant to these herbicides (Pelletier et al 1983).

Cybrid selection in our system is based on screening at the plant level of "recombined" cytoplasmic characters: male fertility (F) and male sterility (S) which are mitochondrial traits and yellowing (Y), normal greening (G) and atrazine resistance (A) which are chloroplastic traits. In fusion experiments, parents differ by both mitochondrial and chloroplastic characters. Several combinations were effected (Table 1) allowing screening of about 20 cybrids.

Table 1: Screening of cybrids among regenerated plants from fusion experiments, according to chloroplastic (ct) and mitochondrial (mt) traits. Y: yellowing, G: normal green. A: Atrazine resistance. F: male fertility, S: male sterility. Ogu, Nap, Cam: Ogura radish, *B. napus*, *B. campestris* cytoplasmic genomes respectively. Ogu/Nap, Ogu/Nap/Cam: recombined mt genomes.

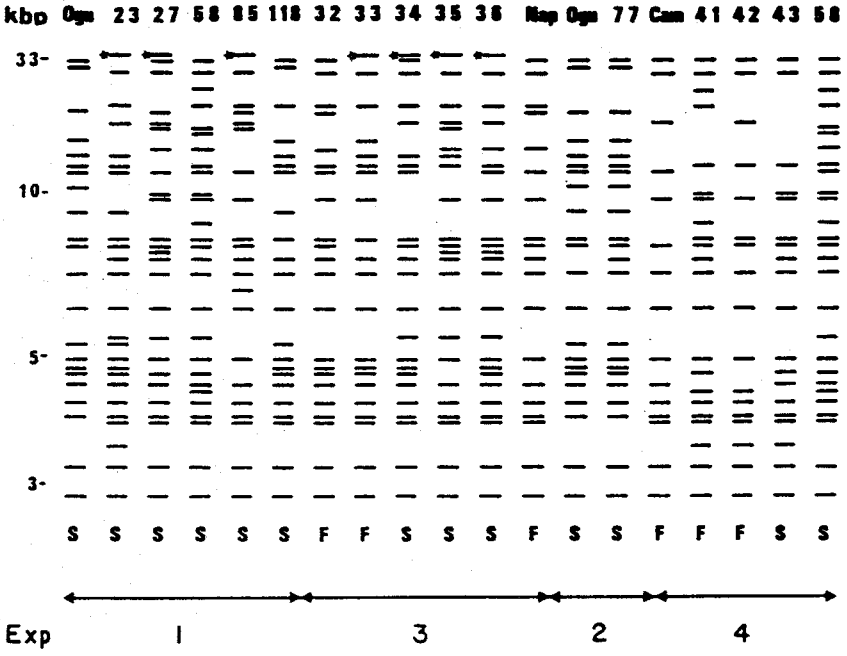
EXPERIMENT	PARENT 1		PARENT 2		EXPECTED CYBRIDS		NUMBER OF CYBRID OBSERVED	CYBRID GENOMES	
	Ct	Mt	Ct	Mt	Ct	Mt		Ct	Mt
1	Y	S(Ogu)	G	F(Nap)	Y	F	0		
					G	S	6	Nap	Ogu/Nap
2	Y	S(Ogu)	A	F(Cam)	Y	F	0		
					A	S	1	Cam	Ogu
3	A (Cybrid 77)	S(Ogu)	G	F(Nap)	A	F	3	Cam	Ogu/Nap
					G	S	4	Nap	Ogu/Nap
4	G (Cybrid 58)	SOgu/Nap	A	F(Cam)	G	F	3	Nap	Ogu/Nap/Cam
					A	S	1	Cam	Ogu/Nap/Cam
5	Y	S(Ogu)	A	F(Ogu/Nap)	Y	F	0		
					A	S	2	Cam	Ogu/Nap

II. MOLECULAR CHARACTERIZATION

Mitochondrial DNA was isolated using CsCl-ethidium bromide gradients (Vedel *et al.*, 1982). Figure 1 shows a diagram of parental (Rap, Nap, Cam) and cybrid (numbers) restriction patterns obtained after agarose gel electrophoresis of mt DNA digests by Sal 1. It is clear from this figure that each cybrid except 77, identical to Rap possesses a new pattern, different from that of its parents and from one another. Novel restriction fragments observed in these cybrids were analysed (Vedel *et al.*, 1986). One of them was chosen to provide molecular evidence that it actually represents intergenomic molecular recombination. This fragment (* on Figure 1), present in several cybrids was found to be made up from recombination of fragment 1 (33 kb) from *B. napus* and fragment 6 (12,3 kb) from *R. sativus*. Sites involved in mt recombination in cybrids were compared by molecular hybridization with sites supposedly implicated in

intragenomic mt recombination. The data showed that intergenomic recombination arising through protoplast fusion involved different mt DNA regions and that some events occurred in regions which are homologous to regions supposed to be involved in natural recombination.

Fig1: SalI mtDNA digests of parental (Ogu, Nao, Cam) and some cybrids corresponding with those obtained in experiments 1, 2, 3, 4 (Table 1). F: male fertile, S: male sterile.



III. PHENOTYPIC CHARACTERIZATION

Male sterile (S) cybrids are of particular interest since agronomical defects previously described (*i.e.* yellowing and absence of nectar production) are now eliminated. Cybrids having *B. napus* or *B. campestris* chloroplast recovered their normal chlorophyll synthesis. Cybrids with *B. campestris* chloroplast are fully resistant to triazines, allowing the use of this cytoplasm in commercial production of resistant hybrid varieties. These traits were stable in sexually produced progeny, no change occuring over following generations.

Variability was found in flower morphology among the cybrids. Nectar production was measured in field conditions for a spring variety (Brutor) with different cytoplasms (*B. napus* and cybrid 27, 58 and 118). Only mitochondria differed in this comparison, since these cybrids and *B. napus* have the same *B. napus* chloroplasts. Nectar production was 81% of that for the fertile control for recombinant n°27, 60% for n° 58, 38% for n° 118, showing that this important agronomical character is controlled by a mitochondria-nucleus interaction (Renard and Mesouida in these Proc.). These observations emphasize the practical importance of mitochondrial rearrangements, since these differences will have an impact on cross pollination by bees and finally on the price of hybrid seeds.

Productivity of Brutor lines having male sterile cybrid cytoplasms was compared to the same variety on its normal fertile cytoplasm, in plots cross pollinated by neighbouring plots (Table 2). It was observed that some cybrid cytoplasms are able to significantly increase productivity of the variety.

Table 2: Comparison of yields of the same line on fertile and male sterile cybrid cytoplasms.

LINE		YIELD (T/Ha)	% CONTROL
CYTOPLASM	NUCLEUS		
58	Brutor	2.81	116
27	Brutor	2.61	108
118	Brutor	2.54	105
B. napus	Brutor	2.41	100

IV. GENETIC CHARACTERIZATION

Generally speaking a cms character is qualitatively defined by a specific genetic (nuclear) system of fertility restoration. The original cms Ogura radish cytoplasm used here was restored by genes existing only in the radish nucleus (Rousselle 1981). They were introduced by sexual crosses into *B. napus* by Heyn (1978). An heterozygous line for these restorer genes was used in crosses with five kinds of cybrids (Table 3). They gave different segregation

Table 3: Segregation of fertility/sterility and flower color in crosses between different cybrids and the same heterozygous restorer. Petal color (W: white, y: yellow) - Male fertility (F: fully fertile, f: some pollen produced, S: sterile). Segregations are similar in crosses with Ogu and cybrid 77 which possess Ogu mtDNA. Frequencies of fully fertile plants in other crosses are significantly higher (34% to 66%). Two phenotypes are not represented (W,S) in the case of cybrid 23 and (y,f) in the case of cybrids 27 and 58. This indicates different causes of sterility in these cybrid.

PHENOTYPE	W, F	W, S	y, F	y, f	y, S	% F
CROSSES						
0 Ogu x 0 RF	14	9	7	15	47	23
0 77 x 0 RF	9	9	3	6	30	21
0 118 x 0 RF	6	10	11	5	17	34
0 27 x 0 RF	4	14	18	0	14	44
0 58 x 0 RF	10	13	11	0	13	44
0 23 x 0 RF	11	0	14	4	11	62

patterns of restoration. In order to interpret these results, taking account of mt DNA recombination as shown by molecular analysis, it must be assumed that Ogura mitochondrial DNA bears more than one cms factor and that cybrids recombinants possess fewer cms factors. The simplest hypothesis is to suppose that "Ogura" mitochondria lead to cms because of two independant determinisms: the "Ogura" male sterility, already expressed in radish, together with an alloplasmic male sterility expressed when radish mitochondria are in the presence of a *Brassica* nucleus (Mc Collum 1981). Mt DNA recombination makes it possible to separate these two factors genetically and to give a simpler system of restoration as in the case of cybrids 27 and 58.

In fact this male fertility restoration system raised an unexpected problem making it useless up till now for hybrid seed production in B. napus. Among segregating progeny of self pollinated restored plants, a decrease in female fertility was found. Male fertile plants were always less female fertile, as though male fertility restorer genes themselves, or radish genes linked to them introduced a female sterility (Pellan Delourme 1986, and in these Proc.).

V. CONCLUSION

Protoplast fusion has been a tool for correcting morphological and physiological defect of radish Ogura cytoplasm. However it seems difficult to use male sterile cybrids in a classical breeding scheme of restored F1 hybrids, because of the low female sterility of restored plants. The production of mixed varieties containing male sterile F1 hybrids and a low proportion of the fertile parents may be an alternative.

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