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Synthesis of *Brassica oleracea*/*Brassica napus* somatic hybrid plants with novel organelle DNA compositions

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Summary. Broccoli (*Brassica oleracea* L. *italica*) hypocotyl protoplasts were fused with mesophyll protoplasts of two *B. napus* lines, one carrying the Ogura (*ogu*) cms cytoplasm, and the other carrying a hybrid cytoplasm consisting of *ogu* mitochondria combined with triazine-tolerance-conferring chloroplasts from *ctr* cytoplasm. Two male-sterile somatic hybrids were recovered from the fusion of broccoli protoplasts with those of *ogu*/*ctr* cybrid *B. napus*. The *ogu* mtDNAs and *ctr* cpDNAs were not altered in these hybrids. Four male-sterile plants were recovered from the somatic hybridization of broccoli with *ogu* cms *B. napus*. Three of these possessed mitochondrial genomes that appeared to have resulted from recombination between the *ogu* and normal *B. oleracea* (*ole*) mtDNAs, while the fourth possessed an unrearranged *ogu* mtDNA. All four of these plants had *B. oleracea* cpDNA, and none displayed the seedling chlorosis associated with *ogu* chloroplasts. Most of the plants recovered from these fusions had the chromosome number expected of *B. oleracea* + *B. napus* hybrids (2n = 56). The novel cytoplasms may prove to be useful for the molecular analysis of *Brassica* cms and for the production of hybrid *Brassica*.

Key words: Cytoplasmic male sterility – Triazine – Ogura mtDNA recombination –Broccoli

Introduction

The genus *Brassica* comprises many important vegetable, oilseed, and condiment crops. For example, the vegetables cabbage, cauliflower, broccoli, and brussels sprouts are all subspecies of *B. oleracea*. *Brassica napus*, *B. juncea*, and *B. campestris* are grown as oilseed crops, while *B. juncea* and *B. hirta* seeds are used in condiment preparation. Much effort has been directed towards the manipulation of cytoplasmic male sterility (cms) systems as a means of large-scale production of *Brassica* hybrids, particularly in the oilseed species (Kemble and Barsby 1988; Braun et al. 1991). A significant component of this effort has involved the expansion of *Brassica* cytoplasmic resources through somatic hybridization approaches. For example, the maternally inherited traits of triazine tolerance (chloroplast DNA encoded) and cms (mitochondrially encoded) have been combined in single cytoplasms (Pelletier et al. 1983; Jourdan et al. 1989b; Kao et al. 1991).

Brassica oleracea hybrid cultivar development has generally employed the use of self-incompatibility (SI) genes found within this species. Nevertheless, production of *B. oleracea* seed that is entirely composed of a desired hybrid is problematic, because the SI conferred by many alleles is incomplete and because maintenance of parental lines requires a method for circumventing the SI. It is possible that cms could be used to augment the weaker, more easily maintainable SI genes of *B. oleracea* and thereby increase the purity of the hybrid seed produced (P. Arnison, personal communication).

Cytoplasmic male sterility has not been extensively investigated in *B: oleracea*. The Ogura or *ogu* cms of radish has been introgressed through sexual crosses into *B. oleracea* (Bannerot et al. 1974), but *B. oleracea* plants with this cytoplasm, like their counterparts in *B. napus*, express chlorosis in seedlings and perform poorly (Bannerot et al. 1977). More recently, protoplast fusion technology has been used to introduce the Polima or *pol* cms of *B. napus* into broccoli (Yarrow et al. 1990). The performance of these plants, however, has not been reported.

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We report here the results of fusions of broccoli hypocotyl protoplasts with mesophyll protoplasts of two *B. napus* strains. Seven plants with unique organelle DNA compositions and phenotypes have been recovered from these experiments. Some of these may prove to be useful sources of cms for *B. oleracea* and/or *B. napus*.

Materials and methods

Plant material

Brassica oleracea ssp. *italica* cv Paragon (Stokes Seed Ltd., St. Catharines, Ontario) was used as the source of hypocotyl protoplasts. Two lines of *B. napus* cv Westar, NFP26-6 carrying an *ogu* cms triazine-tolerant hybrid cytoplasm (Kao et al. 1991), and *ogu* cms Westar carrying the *ogu* cms cytoplasm, were used as sources of mesophyll protoplasts. Plants were maintained as described by Kao et al. (1990).

Protoplast isolation and inactivation

Mesophyll protoplasts were isolated from NFP26-6 and *ogu* cms Westar according to Kao et al. (1991), except that Driselase was omitted from enzyme solution E27. During enzymatic incubation, the digesting leaf tissue was subjected to 100 krad of γ-irradiation from a Caesium 137 source.

Hypocotyl protoplasts were isolated from broccoli cv Paragon as described (Kao et al. 1990). For the purpose of mitotic inactivation, the first protoplast pellet was resuspended to 1% v/v in 1 m*M* iodoacetic acid (IOA) dissolved in a 1:2 mixture of enzyme solution and wash solution (Kao et al. 1990). The protoplasts were maintained in this suspension at room temperature for 30 min, then pelleted by centrifugation (ca. 200 × *g*, 3 min), resuspended in wash solution, and pelleted again by centrifugation. The supernatant was removed and the protoplasts were resuspended in wash solution to obtain a 6–8% v/v suspension. Mesophyll and hypocotyl protoplasts were fused as described by Kao et al. (1991) and cultured through to plant regeneration as described by Kao et al. (1990).

Organelle DNA isolation and analysis

Mitochondrial DNA (mtDNA) was prepared and digested with *Sal*I, *Eco*RI, and/or *Pst*I restriction enzymes (Bethesda Research Laboratories) as described by Kao et al. (1990). Chloroplast DNA (cpDNA) was extracted and analyzed as described by Kao et al. (1991).

Cytology

Metaphases of root-tip cells from regenerated plants and seed-grown Paragon plants were analyzed as described (Kao et al. 1991).

Results

Protoplast fusion and plant regeneration

Protoplasts of the broccoli cultivar Paragon were fused with mesophyll protoplasts of two *B. napus* cv Westar strains: one, NFP26-6, with a hybrid cms cytoplasm consisting of the *ogu* mitochondrial genome in combination with the triazine-tolerance-conferring chloroplast DNA from the *ctr* cytoplasm (Kao et al. 1991), and one with the *ogu* cms cytoplasm (*ogu* cms Westar). Prior to performing protoplast fusion, γ-irradiation and iodoacetate (IOA) selection regimes were developed for the elimination of unfused *B. napus* and *B. oleracea* protoplasts, respectively. Mesophyll protoplasts of *B. napus* cv Westar were found to be extremely resistant to the type of irradiation treatment employed. The 100-krad dose reduced division frequencies by only 63% and colony formation by only 84%, relative to nonirradiated controls. Increasing the dosage beyond this point did not significantly further reduce colony formation. Treatment with 1 m*M* IOA for 30 min was found to kill essentially all unfused *B. oleracea* hypocotyl protoplasts.

For each fusion experiment, several controls were included to determine the effectiveness of the selection scheme: (i) inactivated mesophyll and hypocotyl protoplasts were cultured together without the fusogenic treatment; (ii) inactivated mesophyll protoplasts were cultured together and fused; (iii) inactivated hypocotyl protoplasts were cultured together and fused. The latter two controls tested whether or not the fusogenic treatment would stimulate recovery of the inactivated protoplasts. In controls (i) and (ii), a few colonies (presumably recovered mesophyll protoplasts) developed; in control (iii) all colonies died. No plants regenerated from the colonies recovered from controls (i) and (ii). Thus, it was expected that the plants regenerated from the fusion experiments should all be fusion products.

Several hundred colonies were recovered from the two fusion experiments and nine plants were regenerated (Table 1). All plants except OFP1 grew slower than Paragon regenerants from culture experiments without the fusogenic treatment. Morphologically, the regenerated plants more resembled the broccoli than the *B. napus* fusion partner, in that they had short internodes and large, dark-green leaves (Fig. 1 F). OFP1 had a central compact inflorescence typical of broccoli, while the other plants had inflorescences that were less compact than those of broccoli but more compact than those of *B. napus* cv Westar. The plants all flowered within 2–3 three months after transfer to soil. Although flower morphologies varied among the regenerants, each plant produced only a single characteristic flower type.

Fusion experiment 1

Four plants, designated OFP1, OFP2, OFP6, and OFP7, were regenerated from the protoplast fusion experiment employing broccoli cv Paragon and NFP26-6. OFP1 produced flowers with well-developed anthers that shed abundant pollen (Fig. 1 B), while the flowers of OFP6 and OFP7 showed the *ogu* pistillate male sterility that is characteristic of NFP26-6 (Figs. 1 C–E; Kao et al.1991).

Restriction analysis with *Sal*I (Fig. 2 A) indicated that the mtDNA of OFP1 resembled that of the *B. oleracea*

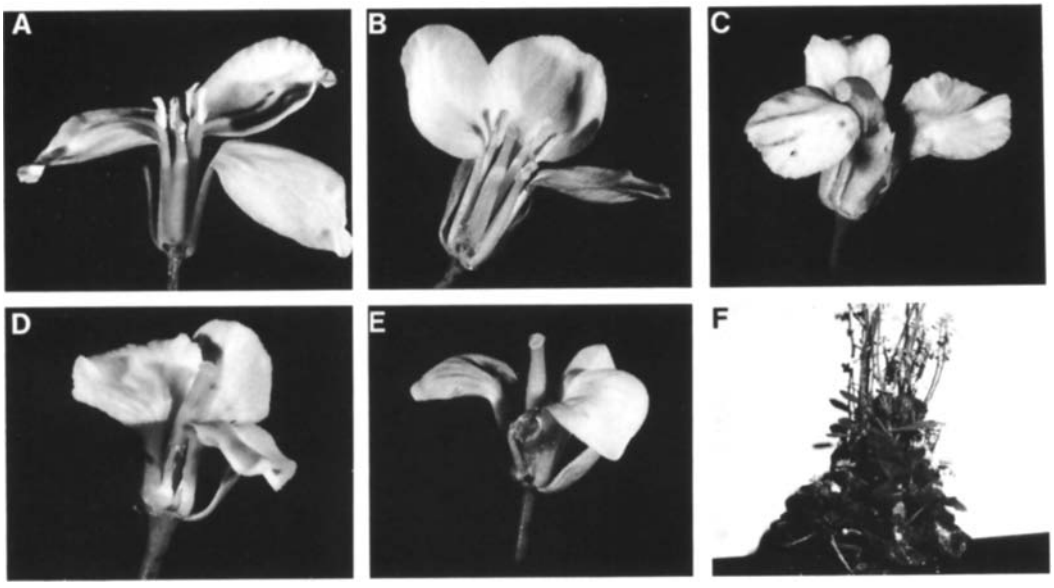

Fig. 1 A–F. Flowers of plants regenerated from fusion 1, between *B. oleracea* cv Paragon and the *B. napus* line NFP26-6. **A** Paragon; **B** OFP1; **C** OFP6; **D** OFP7; **E** NFP26-6; **F** mature fusion product OFP6

Table 1. Characterization of fusion products between *Brassica oleracea* and *B. napus*

Plant	Chromosome number	Petal color[a]	Male fertility[b]	Stamen morphology	mtDNA	cpDNA
Paragon	18	L	F	normal	*ole*	*ole*
NFP26-6	38	Y	S	degenerate/pistillate	*ogu*	*ctr*
ogu cms Westar	38	Y	S	degenerate/pistillate	*ogu*	*ogu*
OFP1[c]	36	I	F	normal	*ole*	*ole*
OFP2	54	I	F	normal	–	–
OFP6	56	Y	S	pistillate	*ogu*	*ctr*
OFP7	56	Y	S	pistillate	*ogu*	*ctr*
OFP8[d]	58	Y	S	reduced	*ogu/ole*[e]	*ole*
OFP9	56	Y	S	reduced	*ogu/ole*	*ole*
OFP11	56	Y	S	reduced	*ogu/ole*	*ole*
OFP13	56	I	S	degenerate	*ogu*	*ole*
OFP14	74	I	S	reduced and dark	*ole*	*ogu*

[a] L – light yellow; I – intermediate yellow; Y – yellow
[b] F – fertile; S – sterile
[c] Fusion products from experiment 1 – OFP1, 2, 6, 7
[d] Fusion products from experiment 2 – OFP8-14
[e] Recombinant *ogu/ole* mtDNA

parent, while OFP6 and OFP7 had the *ogu* mtDNA *Sal*I fragment pattern characteristic of NFP26-6 (Kao et al. 1991). Mitochondrial DNA preparations of NFP26-6 contained a linear DNA plasmid and double-stranded RNAs (Kao et al. 1991). Analysis of undigested mtDNA preparations (Fig. 2B) showed that the dsRNAs, but not the DNA plasmid, were present in OFP6 and OFP7. This suggests that the mitochondrial genome of NFP26-6 was transferred to OFP6 and OFP7 independently of the mtDNA plasmid, as has been observed in other *B. napus* protoplast fusion experiments (Vedel et al. 1987; Kemble et al. 1988; Rosen et al. 1988). *Eco*RI analysis of mtDNA was consistent with the *Sal*I analysis, in that the fragment patterns of OPF6 and OFP7 were those expected of an unrearranged *ogu* mtDNA lacking the linear plasmid (Fig. 3).

*Eco*RI analysis of cpDNA showed that OFP1 had the chloroplast genome of *B. oleracea* (*ole*) cytoplasm, while OFP6 and OFP7 had the chloroplast genome of the *ctr* cytoplasm (Fig. 4). Cytological analysis showed that OFP1 had 36 chromosomes while OFP6 and OFP7 each had 56 chromosomes. Petal color, a nuclear-encoded trait, was the deep yellow of a *B. napus* flower for OFP6 and OFP7 and an intermediate yellow shade for OFP1.

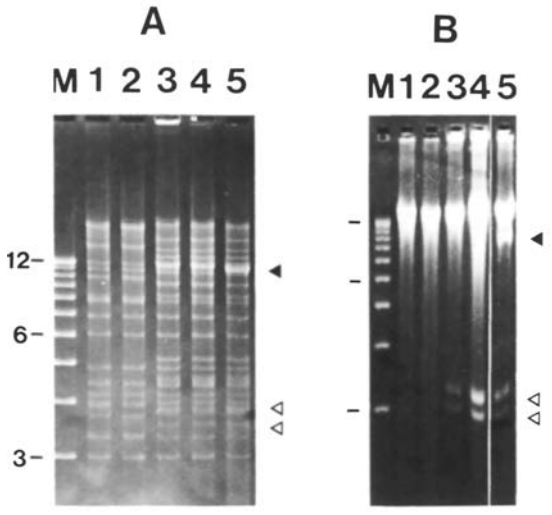

Fig. 2 A and B. *Sal*I-digested (**A**) and undigested (**B**) mtDNA preparations of plants regenerated from fusion 1. Lane *1*, Paragon; lane *2*, OFP1; lane *3*, OFP6; lane *4*, OFP7; lane *5*, NFP26-6; lane *M*, 1-kb ladder marker DNAs. *Closed arrows* (▸) indicate the position of the 11.3-kb linear plasmid DNA; *open arrows* (▹) indicate the positions of the dsRNAs

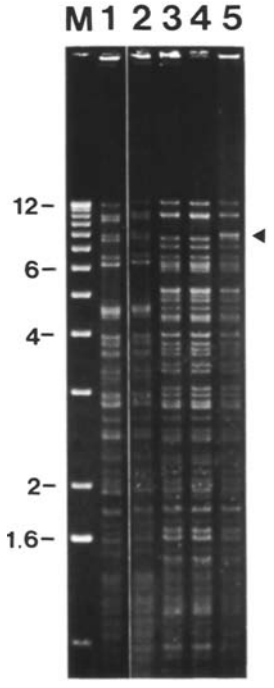

Fig. 3. *Eco*RI-digested mtDNAs of Paragon (lane *1*), OFP1 (lane *2*), OFP6 (lane *3*), OFP7 (lane *4*), and NFP26-6 (lane *5*); lane *M*, 1-kb ladder marker DNAs. *Arrow* indicates the position of the largest *Eco*RI fragment of the linear plasmid DNA

Pistil development was normal for OFP1 and distorted in some, but not all, buds of OFP6 and OFP7.

OFP2 produced flowers with small, normal anthers that shed little pollen. The plant was found to possess 54 chromosomes, but was not sufficiently robust to permit further analysis.

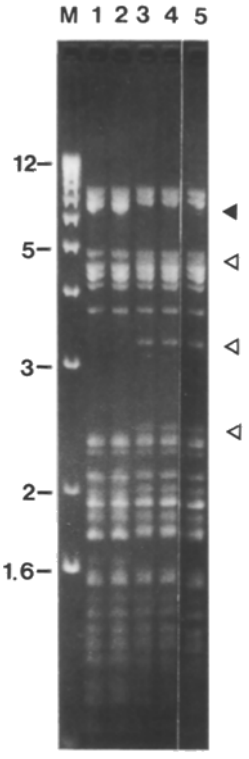

Fig. 4. *Eco*RI-digested cpDNAs of Paragon (lane *1*), OFP1, (lane *2*), OFP6 (lane *3*), OFP7 (lane *4*), and NFP26-6 (lane *5*); lane *M*, 1-kb ladder marker DNAs. *Closed arrow* indicates the position of the fragment specific to *B. oleracea* cpDNA; *open arrows* indicate fragments specific to the *ctr* cytoplasm cpDNA of NFP26-6

Fusion experiment 2

Five plants, designated OFP8, OFP9, OFP11, OFP13, and OFP14, were regenerated from the protoplast fusion experiment employing Paragon and *ogu* cms Westar. All these regenerants expressed male sterility, but varied in the degree of stamen malformation. Stamen development in the regenerants designated OFP8, OFP9, and OFP11 appeared normal in young buds, but arrested upon bud maturation, and resulted in small, sterile anthers (Fig. 5 A–C). Stamen formation in regenerant OFP13 arrested early in bud development (Fig. 5 D).

*Sal*I digests of mtDNA preparations indicated that the mitochondrial genomes of OFP8, OFP9, and OFP11 contained restriction fragments characteristic of both the *ogu* and *ole* mtDNAs, as well as novel fragments not found in either partner, while OFP13 had an unaltered *ogu* mtDNA fragment pattern (Fig. 6). The presence of restriction fragments characteristic of each fusion partner together with novel fragments is thought to indicate that the mitochondrial genome of a regenerated plant has been formed through recombination of the parental mtDNAs (Belliard et al. 1979; Boeshore et al. 1985). The results of *Eco*RI analysis (Fig. 7) were consistent with the view that OFP8, OFP9, and OFP11 all possessed

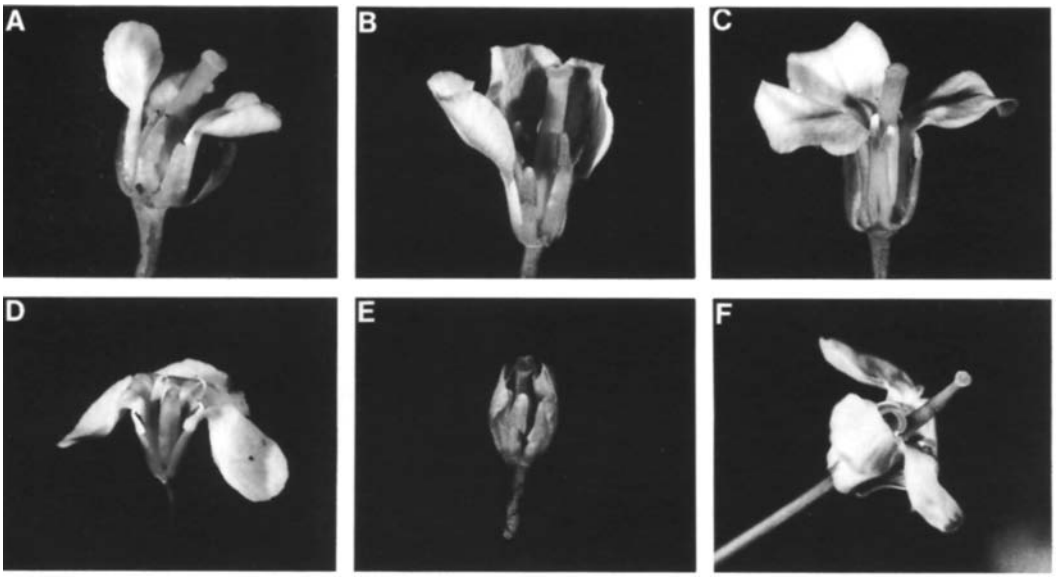

Fig. 5A–F. Flowers of plants regenerated from fusion 2, between Paragon and *ogu* cms *B. napus* cv Westar. **A** OFP8; **B** OFP9; **C** OFP11; **D** OFP13; **E** OFP14; **F** *ogu B. napus* cv Westar

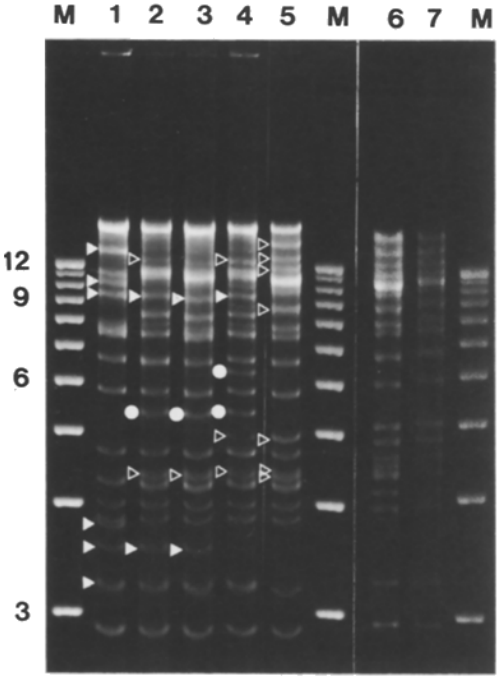

Fig. 6. *Sal*I analysis of mtDNA preparations of plants regenerated from fusion 2. Lane *1*, Paragon; lane *2*, OFP8; lane *3*, OFP9; lane *4*, OFP11; lane *5*, *ogu B. napus* cv Westar; lane *6*, OFP13; lane *7*, *ogu B. napus* cv Westar; lane *M*, 1-kb ladder marker DNAs. *Closed arrows* indicate the positions of fragment specific of *ole* mtDNA; *open arrows* indicate fragments specific to *ogu* mtDNA; *closed circles* indicate novel fragments not found in the mtDNAs of either fusion partner

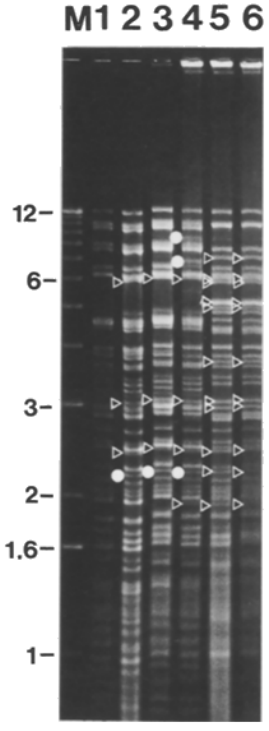

Fig. 7. *Eco*RI analysis of mtDNA preparations of plants regenerated from fusion 2. Lane *1*, Paragon; lane *2*, OFP8; lane *3*, OFP9; lane *4*, OFP11; lane *5*, OFP13; lane *6*, *ogu B. napus* cv Westar; lane *M*, 1-kb ladder marker DNAs. *Open arrows* indicate fragments specific to *ogu* mtDNA; *closed circles* indicate novel fragments not found in the mtDNAs of either fusion partner

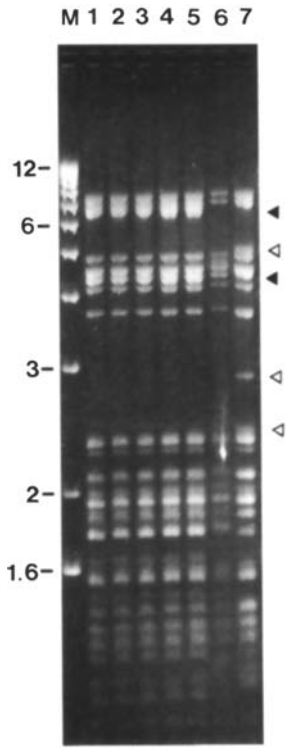

Fig. 8. *Eco*RI-digested cpDNAs of Paragon (lane *1*), OFP8 (lane *2*), OFP9 (lane *3*) OFP11 (lane *4*), OFP13 (lane *5*), OFP14 (lane *6*), *ogu B. napus* cv Westar (lane *7*), lane *M*, 1-kb ladder marker DNAs

recombinant mitochondrial genomes. OFP8 and OFP9 mtDNAs had identical *Eco*RI fragment patterns, characterized by the apparent presence of all of the *ole*-specific fragments as well as *ogu*-specific and novel fragments. OFP11 also appeared to have all the *ole*-specific fragments and additional *ogu*-specific and novel fragments not found in OFP8 and OFP11. In contrast, OFP13 showed an unaltered *ogu* mtDNA *Eco*RI fragment pattern. Analysis of the mtDNAs of OFP8, OFP9, and OFP11 with *Pst*I (not shown) again indicated a recombinant mtDNA pattern consisting of fragments derived from both parents as well as novel fragments. Chloroplast DNA analysis showed that the four regenerants had the *ole* chloroplast genome (Fig. 8).

Cytological analysis showed that OFP8 had 58 chromosomes while OFP9, OFP11, and OFP13 each had 56 chromosomes. Petal color was deep yellow for OFP8, OFP9, and OFP11 and an intermediate yellow for OFP13. Pistil development was generally normal for these plants.

OFP14 was a frail, slow-growing, and morphologically abnormal plant. Its anthers developed normally in young buds, but later degenerated and turned dark. *Eco*RI analysis of organelle DNAs showed that OFP14 had a mtDNA-derived from broccoli (not shown) and an *ogu* cpDNA (Fig. 8). Its pistils were small and underdeveloped and it had 74 chromosomes.

Discussion

The γ-irradiation/IOA selection scheme developed was effective in that the majority of the regenerants represented heterokaryon fusion products. While the γ-irradiation treatment clearly prevented the recovery of unfused *B. napus* mesophyll protoplasts, it apparently did not eliminate the *B. napus* chromosomes; in general, the regenerated plants had a chromosome number expected of *B. oleracea* + *B. napus* somatic hybrids (56) rather than pure *B. oleracea* cybrids (18). The efficiencies of *Brassica* chromosome elimination through γ-irradiation treatment reported in other studies have been variable. Barsby et al. (1987a) and Menczel et al. (1987) found that *Brassica* chromosomes were generally not eliminated by γ-irradiation treatment, while Yarrow et al. (1990) reported highly effective elimination of *B. napus* chromosomes in their *B. oleracea* – *B. napus* fusions.

The plants OFP6 and OFP7 recovered from the first fusion experiment, between broccoli cv Paragon and the *ogu* CMS, triazine-tolerant *B. napus* cybrid NFP26-6, are judged to be *B. oleracea* + *B. napus* somatic hybrids on the basis of their chromosome number and morphological characteristics. They carry the *ogu* cytoplasm mitochondrial genome and the cpDNA of *ctr* cytoplasm. Correspondingly, the plants have the characteristic Ogura cms phenotype and are expected to show tolerance to triazine herbicides. To our knowledge, this experiment is the first detailed description in the *Brassicaceae* of the use of a previously synthesized cybrid as a fusion partner with protoplasts of a different species. It sould be possible to use these plants as bridging genotypes for the development of cms triazne-tolerant *B. oleracea*.

The fertile plant OFP1 had 36 chromosomes and resembled broccoli in every respect, except for its larger and deeper yellow flower petals. OFP1 is most probably a tetraploid *B. oleracea*, possibly resulting from a *B. oleracea* – *B. oleracea* fusion event.

In the second fusion experiment, involving Paragon and *ogu* cms Westar, regenerants OFP8, OFP9, and OFP11 were found to possess recombinant mtDNAs, and regenerant OFP13 to possess an unaltered *ogu* mtDNA. The four regenerants were cytoplasmic hybrids since they also possessed the *ole* cpDNAs. As a result, the plants were a dark-green color and did not suffer from the chlorosis and poor nectary development that characterize *ogu* cms *B. oleracea* (Bannerot et al. 1977). Pelletier et al. (1983) previously observed that the replacement of the *ogu* radish chloroplast with *nap* chloroplasts in *ogu* cms *B. napus* remedied the incompatibility of the *ogu* cms. The plants are most probably *B. oleracea* + *B. napus* fusion products, since three out of four had the characteristic nuclear-encoded yellow petal color of the *B. napus* parent and three out of four had 56 chromosomes, representing the sum of the addition of the *B.*

oleracea (18) and *B. napus* (38) chromosomes. OFP13 had a slightly lighter yellow petal color and OFP8 had two more chromosomes than the expected 56. Reasons for these variations are unknown.

The last regenerant obtained, OFP14, also had a unique hybrid cytoplasm consisting of the *ole* mtDNA and the *ogu* cpDNA. Although not of agronomic value, the recovery of this organelle combination is interesting. OFP14 had 74 chromosomes suggesting that it derived from a fusion of one *B. napus* and two *B. oleracea* genomes.

The three regenerants with recombinant mtDNAs may prove useful in identifying the mtDNA determinants that contribute to the Ogura male-sterile phenotype. Moreover, these plants may represent new and potentially useful forms of cms. Although the stamens on these plants are reduced and completely sterile, the plants lack the more pronounced flower malformations and other undesirable characteristics of the Ogura cms. The expression of the cms trait in *B. napus* plants into which these cytoplasms have been introduced through backcrossing should prove particularly interesting. Nuclear restoration of male sterility is required for a workable cms system in oilseed crops such as *B. napus*. It may be that the male sterility conferred by these recombinant mtDNAs is altered such that some of the difficulties associated with the development of Ogura cms restorer lines (Pellan-Delourme and Renard 1988) are circumvented. Evidence for mtDNA recombinations associated with easier restoration of the cms trait has been observed in *B. napus nap/ogu* cybrids (Pelletier et al. 1988).

It is unlikely that either the male sterility or novel mtDNA patterns observed in the regenerants were induced by the protoplast culture system, since regenerants obtained from broccoli protoplast culture without fusion were male fertile and lacked mtDNA rearrangements (Kao et al. 1990). The possibility that unique mtDNA fragment patterns resulted from partial digestion is also unlikely, since similar fragment patterns were obtained even after overnight digestion. In addition, the parental *Sal*I and *Eco*RI fragment patterns were identical to those reported by others (Chetrit et al. 1984; Kemble 1987; Vedel et al. 1987; Palmer 1988). Mitochondrial DNA digests of OFP11 routinely showed the presence of a few additional faint bands, suggesting that this plant lineage may remain heteroplasmic for mtDNA and that sorting out of may be ongoing.

The different combinations of parental organelles observed in the relatively small number of regenerants recovered in this study indicate that the organelles tended to sort out randomly following protoplast fusion. In contrast, most previous studies have reported nonrandom sorting out in *Brassica* protoplast fusion experiments (Pelletier et al. 1983; Yarrow et al. 1986; Morgan and Maliga 1987; Chuong et al. 1988; Jourdan et al. 1989a, b). Our results do not support the hypothesis that the organelles in fusion products tend to remain in the combinations of the parental plants (Barsby et al. 1987b).

Previous reports have indicated that either the *ogu* mitochondrial genome undergoes extensive rearrangement (Vedel et al. 1986, 1987; Menczel et al. 1987; Morgan and Maliga 1987; Robertson et al. 1987; Jourdan et al. 1989a) or that it remains unaltered (Kemble et al. 1988; Jourdan et al. 1989b) during protoplast fusion/regeneration experiments. Interestingly, both situations occurred in the second fusion experiment, where regenerants with both recombinant and unaltered *ogu* mitochondrial genomes were recovered in the same experiment.

Plants with unique organelle DNA compositions have been recovered from these experiments. Five of the nine regenerants have hybrid cytoplasms that cannot be produced by conventional backcrossing procedures. It is unlikely that the hybrid nuclear genetic composition of these plants caused the male sterility, since sexually produced *B. napus* + *B. oleracea* hybrids are male fertile (Quazi 1988). Nevertheless, to verify the cause of the male sterility, these cytoplasms should be incorporated into a normal nuclear background. Initial backcrosses of these regenerants to either *B. oleracea* or *B. napus* led to vigorous silique formation for the first 2 weeks following pollination, after which normal seed development was generally blocked. Fertilization of OFP11 with *B. napus* pollen, however, led to the formation of a few small seeds, and it has been possible to induce one of these to germinate. By employing the ovule culture technique of Ripley and Arnison (1990), it may be possible to obtain plants from the other crosses as well.

To our knowledge, this is the first detailed report of the synthesis of cytoplasms in which the *ogu* mtDNA is combined with the *ole* cpDNA. It is, in addition, the first report of *ogu/ole* recombinant mtDNAs. Only Yarrow et al. (1990), who transferred the Polima cms from *B. napus*, have previously reported on the improvement of cms systems in *B. oleracea* through somatic hybridization. Kameya et al. (1989) have synthesized a male-sterile *B. oleracea* through fusion with radish protoplasts, but the plants also carried the incompatible radish chloroplast genome.

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