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Atrazine-resistant cytoplasmic male-sterile-*nigra* broccoli obtained by protoplast fusion between cytoplasmic male-sterile *Brassica oleracea* and atrazine-resistant *Brassica campestris*

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Summary. Protoplast fusion was used to combine the cytoplasmic traits of atrazine resistance and male sterility in *Brassica oleracea* var. *italica* (broccoli). Leaf protoplasts from broccoli with the petaloid *B. nigra* type of cytoplasmic male sterility were fused with hypocotyl protoplasts from an atrazine-resistant biotype of *B. campestris* var. *oleifera* cv Candle (oilseed rape). A total of 19 colonies regenerated shoots, all of which were broccoli-like in phenotype, i.e., lacked trichomes. Four shoots, all from one colony, were atrazine resistant, surviving and growing in the presence of 25 μM atrazine. A leaf piece assay also confirmed that they were atrazine resistant. Molecular analysis showed that they contain chloroplasts from the atrazine-resistant *B. campestris* parent and mitochondria from the *B. nigra* parent. No recombination or rearrangement of the mitochondrial genomes in the fusion products was detected. These four plants and their progeny all showed the petaloid *B. nigra* type of male sterility.

Key words: *Brassica* – Atrazine resistance – Cytoplasmic male sterility – Protoplast fusion – CMS-*nigra*

Introduction

In *Brassica oleracea*, there are two types of alloplasmic cytoplasmic male-sterile (cms) systems, based on either the *Raphanus sativus* cytoplasm (cms-Ogura; Bannerot et al. 1974), or the *B. nigra* cytoplasm (cms-*nigra;* Pearson 1972; Dickson 1975). Cms-Ogura lines are characterized by small anthers with no pollen grains (Bannerot et al. 1974), whereas cms-*nigra* lines have petaloid stamens (Pearson 1972). Cms-Ogura *B. oleracea* lines exhibit chlorosis at low temperatures (Bannerot et al. 1977) as well as lower yield and vigor than fertile lines (Hoser-Krauze and Antosik 1987). These defects need to be corrected before cms-Ogura can be used for hybrid seed production. In contrast, cms-*nigra B. oleracea* lines do not have the disadvantages of the cms-Ogura system. They do not show chlorosis at low temperatures (M. C. Christey, unpublished observations) and some lines show good seed set (Dickson and Kyle 1987).

It would be desirable to combine the cytoplasmic characters of cms and atrazine resistance (ATR) in broccoli. Cms would be of use in hybrid seed production, and the presence of ATR would allow growth on soils already containing atrazine residue. Also, these two characters could be used in combination in hybrid seed production to eliminate pollinators after fertilization (Beversdorf et al. 1985). Because maternal inheritance of the *Brassica* cytoplasm prevents organelle segregation, these cytoplasmic traits can be combined only by protoplast fusion and not by conventional breeding methods. Previously, protoplast fusion has been used in the Cruciferae to produce intergeneric (Chatterjee et al. 1988) and interspecific (Schenck and Röbbelen 1982; Glimelius et al. 1986; Jourdan et al. 1989 b) somatic hybrids. Protoplast fusion has also been used to manipulate cytoplasmic traits. In *B. napus,* protoplast fusion has enabled the combination of cms (Ogura, Polima, or *nap*) with ATR (Pelletier et al. 1983; Yarrow et al. 1986; Chuong et al. 1988) and replacement of the *R. sativus* chloroplasts in Ogura lines (Jarl and Bornman 1988). In *B. oleracea*, protoplast fusion involving cytoplasm manipulation has allowed the production of ATR fertile cauliflower (Jourdan et al. 1989 a), replacement of the *R. sativus* chloroplasts in

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Ogura lines (Pelletier 1989), transfer of Polima cms to broccoli (Yarrow et al. 1990), and production of cold-tolerant cms cauliflower (Walters et al. 1990).

This study reports the successful combination of two cytoplasmic traits, ATR and cms-*nigra* in broccoli. Protoplasts from a cms-*nigra* broccoli line were fused with protoplasts from an ATR line of *B. campestris*. Four ATR-cms-*nigra B. oleracea* plants were obtained in the absence of any selection or pretreatment. These plants exhibit petaloid sterility and are female fertile. Progeny obtained after crosses with maintainer lines are also petaloid male sterile and are as vigorous as atrazine sensitive (ATS) cms-*nigra B. oleracea* under field conditions (in preparation).

Materials and methods

Plant material

The original cms-*nigra* broccoli plant (BN no. 5) was obtained from M. H. Dickson (New York State Agricultural Experimental Station, Geneva/NY). This plant has the nuclear background of Green Comet broccoli and contains the *B. nigra* cytoplasm. Further plants were obtained from BN no. 5 by culturing longitudinal stem explants on Linsmaier-Skoog (LS) medium (Linsmaier and Skoog 1965) containing 3% sucrose, 1 mg/l naphthalene acetic acid, and 12.5 mg/l benzyladenine, solidified with 0.25% Gelrite (Scott Laboratories Inc.). The particular stem explant regenerant used in this study was designated BN no. 5C-J. The ATR *B. campestris* line used was an ATR biotype of the cultivar Candle (Beversdorf et al. 1980) obtained from W. D. Beversdorf (University of Guelph, Ontario, Canada). Pollinators for the fusion-derived plants (broccoli lines known to maintain the cms-*nigra*) were obtained from M. H. Dickson.

Protoplast isolation, fusion, and culture

Leaf mesophyll protoplasts from plant BN no. 5C-J were fused with etiolated hypocotyl protoplasts from ATR *B. campestris*. Mesophyll protoplasts were isolated from semiexpanded leaves of cuttings of BN no. 5C-J maintained in vitro by monthly subculture on hormone-free LS medium. Cultures were kept at 25 °C under 16-h photoperiod with lighting provided by equal numbers of Cool White and Gro and Sho fluorescent lights, 45–70 $\mu E/m^2/s$. For the isolation of hypocotyl protoplasts, seeds were surface sterilized and cultured in the dark at 25 °C for 6 days on hormone-free LS medium.

The procedure used for protoplast isolation was based on that developed by Robertson and Earle (1986), with 0.2 *M* mannitol and 80 m*M* $CaCl_2 \cdot 2H_2O$ (M + C) in place of SCM. The final protoplast pellet was resuspended in M + C prior to fusion.

For fusion, protoplasts from each source were resuspended in 100 µl of M + C, mixed together as a single droplet in a 35 mm petri dish, and allowed to settle for 20 min. Then 50 µl of a solution containing 33% polyethylene glycol (MW 3350, Sigma), 0.2 *M* glucose, 10 m*M* $CaCl_2 \cdot 2H_2O$, 5 m*M* MES, and 0.7 m*M* KH_2PO_4 (pH 6.0), was added as several small droplets to the edge of the settled protoplast mixture, followed by 200 µl of a solution containing 0.5 *M* sorbitol, 100 m*M* $CaCl_2 \cdot 2H_2O$, and 5 m*M* MES (pH 7.0), after 25 min and then again after a further 20 min. The excess liquid was removed with a pasteur pipette 10 min later, and the plate was flooded with 5 ml of medium B (Pelletier et al. 1983).

After fusion, protoplasts were cultured using the medium sequence developed by Pelletier et al. (1983), with some modification. Solid media were solidified with 0.25% Gelrite. In medium B, Tween 80 and Gentamycin were omitted, and the level of $CaCl_2 \cdot 2H_2O$ used was 150 mg per liter. Medium D was not used, and GA_3 was omitted from medium E.

Plates were cultured in the dark at 25 °C for 10 days and then transferred to a 16-h photoperiod, 45–70 $\mu E/m^2/s$. Approximately 0.5 ml of medium C was added to the plate after day 10 and then every 5 days. After 3 weeks, when large green colonies were present, excess liquid was removed from the plate and the remaining contents were plated onto solid medium E. After 3 weeks on medium E, colonies were transferred to medium F for shoot regeneration. Shoots were then transferred to hormone-free LS medium for elongation and rooting.

Determination of resistance or sensitivity to atrazine

Two assays for resistance to atrazine were used: (1) cutting assay, and (2) leaf piece assay. In the cutting assay, small, green, healthy cuttings consisting of the apical meristem and one visible folded leaf were placed onto LS medium containing 0.3% sucrose and 25 µ*M* atrazine. Atrazine was added to autoclaved medium from a 50 m*M* stock solution in 95% ethanol and stored at −20 °C. Cuttings (one to seven) from each colony that regenerated plants were tested on this medium and also on standard LS medium (3% sucrose, no atrazine). As controls, cuttings from ATS Green Comet broccoli and ATR Candle seedlings were placed onto LS media (0.3% sucrose) with and without atrazine (25 µ*M*). Results were determined after 2 weeks.

For the leaf piece assay, leaf pieces (0.5 cm^2) from in-vitro-grown cuttings of BN no. 5C-J and fusion regenerants were cultured on medium F containing 0, 50, or 100 µ*M* atrazine.

Floral characteristics of regenerated plants

Plantlets with good root and shoot development were transferred to soil and covered with a plastic bag to maintain humidity. After 1–2 weeks, holes were gradually cut in the bag, which was removed after a further week. Plants were initially grown under a 16-h photoperiod with lighting provided by Cool White fluorescent lights, 35 $\mu E/m^2/s$, 25 °C. After 3 weeks, plants were transferred to the greenhouse, where lighting was daylight-supplemented with Cool White fluorescent lights, 100 $\mu E/m^2/s$. Flowers that appeared were examined for petaloid stamens.

Isozyme analysis

Extracts were obtained from approx. 100 mg of leaf tissue from six fusion regenerants from three colonies and from the Candle fusion partner. Isozyme analysis was conducted using cellulose acetate electrophoresis following the manufacturer's instructions (Helena Laboratories, Beaumont, TX), except that the extraction buffer contained 0.1% B-mercaptoethanol and 0.5% Triton X-100 in 0.1 *M* potassium phosphate, pH 7.0 (Wendel and Weeden 1989). The isozymes of phosphoglucose isomerase (PGI) and phosphoglucosomutase (PGM) were tested.

Southern analysis

Total DNA was isolated from 16 fusion regenerants (obtained from seven colonies), the two fusion partners (Candle and BN no. 5C–J), and five regenerants from BN no. 5 protoplasts, using the procedure of Dellaporta et al. (1985). Mitochondrial DNA (mtDNA) was isolated from four of the fusion-derived plants and the two fusion partners using the procedure of Kolodner and Tewari (1972).

Total DNA and purified mtDNAs were digested with *BglI* and *PstI*, respectively, separated on 0.7% agarose gels, and transferred to Zetabind (AMF Cuno) membranes using stan-

Table 1. Analysis of plants regenerated after protoplast fusion

Colony designation	No. of shoots regenerated	Atrazine R/S[a]	Plants potted	cpDNA analysis[b]	Fertile/sterile[c]
2	3	S	0	ND	ND
3	2	S	0	ND	ND
4	6	S	1	N	sterile
5	13	S	2	N	sterile
7	4	S	5	N	sterile
12	4	S	2	N	sterile
13	9	S	2	N	sterile
14	4	S	1	N	sterile
15	1	S	0	ND	ND
16[d]	4	R	4	C	sterile
17	1	S	0	ND	ND
18	2	S	0	ND	ND
19	4	S	0	ND	ND

[a] R = resistant, S = sensitive, as determined by cutting assay and/or leaf assay
[b] N = *Brassica nigra*, C = *B. campestris*, ND = not determined
[c] Sterile = petaloid stamens, fertile = normal stamens, ND = not determined
[d] Desired cybrid combination

dard procedures (Maniatis et al. 1982). Probes used in the hybridization experiments were labeled by nick-translation and are described in the figure legends.

Results

Protoplast fusion

Although 25 fusion experiments were conducted, plant regeneration was obtained only in 1. In the successful experiment, BN no. 5C-J yielded 1.4×10^7 leaf protoplasts per gram of fresh weight. The yield of Candle hypocotyl protoplasts was very low. The fusion droplet contained 2×10^4 BN no. 5C-J protoplasts and approximately the same number of Candle protoplasts. Immediately after fusion, protoplasts appeared intact with high survival. After 3 weeks, numerous green healthy colonies, often with elongated cells, were present. After plating onto medium E good colony formation was noted, with the plating efficiency estimated to be 1% of the original protoplasts. Of these colonies, 19 regenerated shoots (one to seven per colony) on medium F. All these plants were broccoli-like in phenotype, lacking trichomes characteristic of *B. napus* and *B. campestris*, and were therefore either escapes or cybrids.

Determination of resistance or sensitivity to atrazine

Several methods were used to determine whether or not any of the plants regenerated from the protoplast fusion experiment were atrazine resistant and therefore cybrids. Shoots from 6 of the 19 colonies that regenerated shoots could not be assayed because they did not survive. The remaining 13 colonies produced four atrazine-resistant plants, all from one colony (no. 16) (Table 1). Both the leaf piece and cutting assays supported this conclusion. Analysis of photosynthetic activity in isolated protoplasts with nitro-blue tetrazolium (Robertson and Earle 1987) also provided results consistent with these assays, but was less satisfactory than in Robertson and Earle (1987).

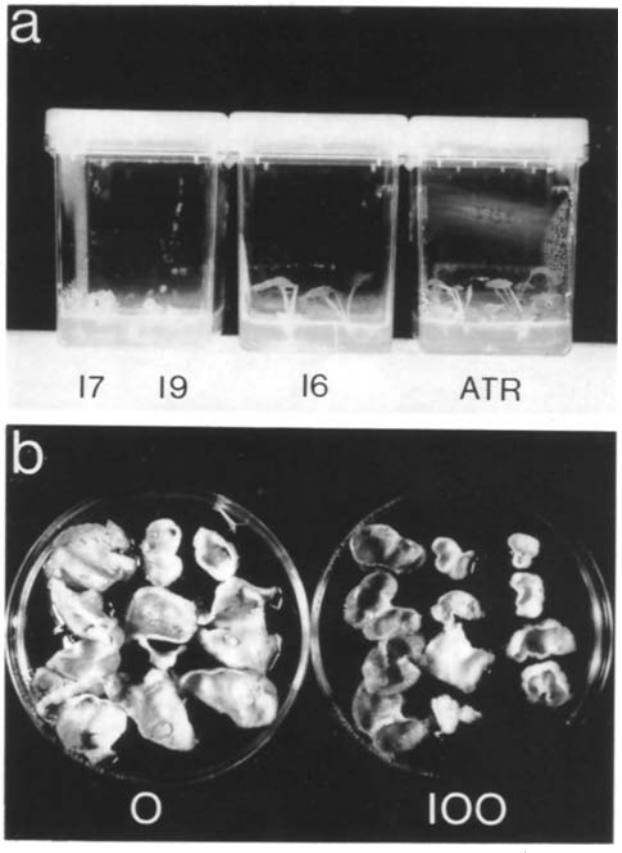

Fig. 1 a and b. Determination of resistance or sensitivity to atrazine. **a** Cutting assay, 2 weeks after culture. Shoot cuttings from ATR Candle and from three fusion-derived colonies (no. 16, no. 17, and no. 19) were placed onto media containing 25 μM atrazine. **b** Leaf piece assay, 3 weeks after culture. Leaf pieces were cultured on medium F containing O or 100 μM atrazine. On each plate, from *left* to *right*, are leaf pieces from shoots from colony no. 16 and no. 7 (both fusion-derived) and from BN no. 5C-J, the ATS fusion partner

1. Cutting assay. After 2 weeks, ATS cuttings grown on medium with 25 μM atrazine were small and pale green or yellow, with little shoot growth and no root regeneration (Fig. 1 a). Cuttings from 12 of the protoplast colonies and from Green Comet showed this response. In contrast, cuttings from the ATR line (Candle) and the four plants from protoplast colony no. 16 were green elongated, and rooted (Fig. 1 a). They appeared no different from cuttings grown in the absence of atrazine.

2. Leaf piece assay. This assay was easier to conduct and also provided convincing proof that the four plants from colony no. 16 were atrazine resistant. After 7 days on medium F containing 50 μ*M* or 100 μ*M* atrazine, there was little difference between the various lines: all explants were still dark green. After 10 days, leaf pieces from ATS lines were a slightly paler green than the others. After 3 weeks on medium F containing either 50 μ*M* or 100 μ*M* atrazine, the differences between the ATR and ATS lines were very clear (Fig. 1 b). Explants from shoots from colony no. 16 were green and enlarged on both media, with small amounts of green callus in a few areas. In contrast, leaf pieces from Green Comet and other ATS lines were small and pale brown, with only small patches of greening. On medium F with no atrazine, leaf pieces from all sources remained green and enlarged, with small amounts of green callus on the edges of explants.

Fig. 2 a and b. Flower structure **a** Flower with petaloid stamens from the ATR-cms-*nigra* cybrids. **b** Flower with normal stamens from fertile broccoli

Floral characteristics of fusion regenerants

A total of 17 plants derived from seven colonies from this protoplast fusion experiment were transferred to soil. The four atrazine-resistant plants from colony no. 16 exhibited petaloid sterility (Fig. 2), indicating that they are cybrids. These plants are vigorous and appear morphologically identical to other cms-*nigra* broccoli. Of the remaining 13 ATS plants, the 10 that flowered were petaloid sterile, confirming that they were escapes. Had they also been cybrids, they would have been male fertile with normal stamens. All progeny from colony no. 16 (25 plants) obtained after pollination with maintainer lines were vigorous (Fig. 3) and were also petaloid cms.

Isozyme analysis

All fusion regenerants tested had the same isozyme patterns, which were different from that of Candle (data not shown). Four of the six fusion regenerants tested were the ATR-cms cybrids from colony no. 16. Thus the nuclear content of these ATR-cms cybrids is the same as the ATS escapes but distinct from Candle, at least for the two isozymes examined.

Fig. 3. Growth of ATS-cms-*nigra* (*left*) and ATR-cms-*nigra* broccoli (*right*) seedlings 6.5 weeks after planting seed

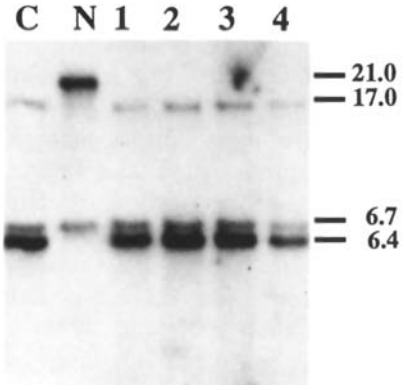

Fig. 4. Analysis of chloroplast DNA in ATR-cms fusion products. A Southern blot containing *BglI* restriction digests of total DNAs isolated from BN no. 5C-J (*N*), *B. campestris* Candle (*C*), and four ATR-cms fusion products (*1–4*) was hybridized with *Petunia* cpDNA fragment S8. Sizes of fragments identified are shown in kb at the right

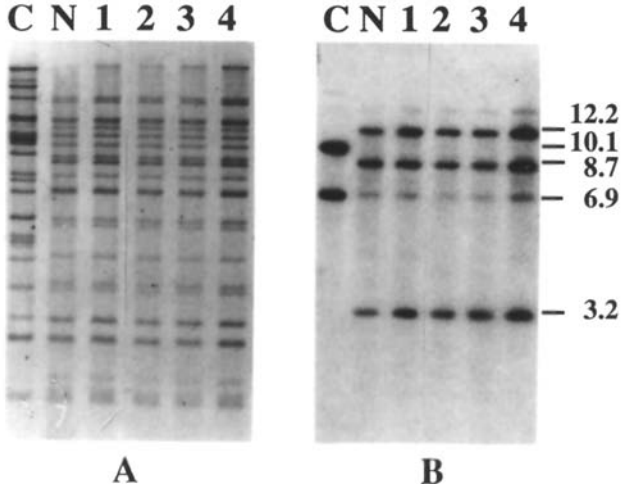

Fig. 5. Analysis of mitochondrial DNA in ATR-cms fusion products. *PstI* restriction digests of purified mtDNAs isolated from BN no. 5C-J (*N*), *B. campestris* Candle (*C*), and four ATR-cms fusion products (*1–4*) were resolved on a 0.7% agarose gel (Panel *A*), transferred to Zetabind filters, and probed with *B. campestris* mtDNA clones P 6.9 and P 10.1 (Panel *B*). Sizes of the hybridization products are shown in kb at the right. See Palmer and Herbon (1988) for restriction maps and a comparison of the *B. nigra* and *B. campestris* mitochondrial genomes

Organellar genome analysis

The identity of the chloroplast genome was confirmed by Southern analysis using an 8.0-kb *Sacl* fragment of *Petunia* chloroplast DNA (cpDNA). This fragment hybridizes to *BglI* fragments of 6.4, 6.7, and 17 kb in the ATR-*B. campestris* cpDNA, and fragments of 6.7 and 21 kb in the *B. nigra* cpDNA (Fig. 4). The four ATR-cms plants exhibited the *B. campestris* hybridization pattern, confirming that they all contain the ATR-Candle chloroplasts.

Purified mtDNA, isolated from four of the ATR-cms plants and the two parental lines, was compared by restriction and Southern analysis. Examples of the results obtained from these experiments are shown in Fig. 5. All four of the ATR-cms plants exhibited the same mtDNA restriction profile as the *B. nigra* parent (Fig. 5A). In order to determine whether the fusion products contained an intact *B. nigra* mitochondrial genome or if mtDNA rearrangements occurred, as often happens during protoplast fusion, the mtDNAs were analyzed further. Clones that identified approximately 95% of the *B. campestris* and *B. nigra* mitochondrial genomes were used as hybridization probes against Southern blots containing the two parental and the four ATR-cms mtDNAs. The four ATR-cms lines contained only *B. nigra* mtDNA fragments (Fig. 5B): no *B. campestris* or novel fragments were observed. This confirms that mitochondria in the fusion products were derived from the *B. nigra* parent and indicates that recombinant mitochondrial genomes were not generated during protoplast fusion.

Discussion

This work represents the first use of cms-*nigra* lines in protoplast fusion and has described the successful production of ATR-cms-*nigra* broccoli plants by protoplast fusion.

Of the 12 possible outcomes after fusion of the protoplast sources used, we were most interested in 1. This involved no nuclear fusion but cytoplasm mixing and reassortment to produce a broccoli nuclear background, with ATR from *B. campestris* and retention of the *B. nigra* cms. It is possible to eliminate some of the other outcomes by various treatments. For example, irradiation (Menczel et al. 1987), irradiation combined with chemical inactivation with iodoacetate (Barsby et al. 1987a), or rhodamine 6G and iodoacetate treatment (Jourdan et al. 1989b) have been used to prevent divisions of one or both of the fusion partners. In other examples, survival on atrazine-containing media has been used to select cells with ATR chloroplasts (Jourdan et al. 1989a). Irradiation does not necessarily prevent the contribution of nuclear DNA from the irradiated partner, as Menczel et al. (1987) obtained a plant that had a doubled chromosome number even though irradiation was used. However, chromosome doubling could have occurred during culture. There is some evidence that inactivation with iodoacetate or rhodamine 6G can affect the organellar composition of the cybrids, favoring a particular combination (Galun et al. 1987). For these reasons, no pretreatment or selection was used in these experiments until shoots were regenerated. One disadvantage of this approach is that large numbers of plants may have to be assayed; however, this was not a problem

due to the low regeneration obtained. Of the possible outcomes from fusing *B. oleracea* and *B. campestris* protoplasts, *B. campestris* escapes and resynthesized *B. napus* hybrids can be distinguished and eliminated because they have trichomes, which are absent in broccoli. Only broccoli-like plants were obtained, however. These were then tested for their ability to grow on atrazine-containing media.

No *B. campestris* plants were expected due to the low totipotency of this material under the culture conditions used (M. C. Christey, unpublished observations). The lack of somatic hybrids was surprising, however. The fusion and culture conditions should permit *B. napus* regeneration, as the medium sequence used was based on that developed for *B. napus* (Pelletier et al. 1983). It is possible that some *B. campestris* chromosomes or parts thereof were introduced during fusion. Chromosome counting would not be sensitive enough to detect transfer of a portion of the *B. campestris* genome. Isozyme analysis revealed no *B. campestris* isozymes of PGM or PGI. The lack of somatic hybrids contrasts with the results of Jourdan et al. (1989a), who obtained only somatic hybrids after fusing *B. oleracea* and *B. campestris* protoplasts. They suggested that the nuclear background may affect nuclear and organellar behavior, but as their fusions involved chemical inactivation of one or both partners and selection on atrazine, it is likely that their fusion and selection conditions favored a particular nuclear and organellar combination.

In the present study, the cybrid combination of ATS with fertility was not obtained, suggesting nonrandom organellar segregation, even in the absence of pretreatment and selection. In other protoplast fusions in *B. napus* involving cytoplasm manipulation, evidence has also suggested that not all combinations are equally favored. It is possible that nuclear control is involved, or perhaps the in vitro conditions favor a particular combination. To determine the mechanism more clearly, a study of many more cybrids is required, as in most published experiments fewer than ten plants were analyzed. Pelletier et al. (1983) did not obtain male-fertile plants with Ogura chloroplasts. Chuong et al. (1988) also used no selection; out of 261 regenerants only 1 was the desired combination of ATR-cms, and the remainder were parental types. No ATS-fertile plants were obtained. Yarrow et al. (1986) used selection, but found that organellar sorting appeared to favor the native *B. napus* chloroplasts (ATS). Barsby et al. (1987b) also noted nonrandom organellar sorting as they obtained no ATS-fertile plants. The type of cell used in the fusion does not appear to affect the outcome. Barsby et al. (1987b) found that mitochondria were not contributed from mesophyll protoplasts, and in the present study mitochondria were not contributed from hypocotyl protoplasts. In the present study, proplastids from the hypocotyl protoplasts were able to out-compete the chloroplasts from the mesophyll protoplasts. This has been reported previously in other fusions involving *Brassica* (Barsby et al. 1987a; Jourdan et al. 1989a, b; Yarrow et al. 1990).

Detailed characterization of the organellar genomes in the ATR-cms-*nigra* fusion products indicates that the chloroplasts were contributed by the *B. campestris* parent and the mitochondria were contributed by the *B. nigra* parent. Thus, protoplast fusion and chloroplast transfer has occurred, rather than a mutation. Culture-induced triazine resistance is limited to one report with *Nicotiana plumbaginifolia;* the resistance was maternally inherited (Cseplo et al. 1985).

No recombination or rearrangement of the mitochondrial genome was detected in the ATR-cms fusion products. The presence of such mtDNA recombination may have been of use in identifying the region associated with cms-*nigra,* as has been achieved in *Petunia* (Young and Hanson 1987). In other studies involving cytoplasm manipulation in *Brassica,* both mtDNA recombination (Morgan and Maliga 1987; Jourdan et al. 1989b) and lack of mtDNA recombination (Jourdan et al. 1989a; Yarrow et al. 1990) have been reported.

The ATR-cms plants developed may be of particular use for horticultural purposes. They may be useful for hybrid seed production after further selection to remove the undesirable characters, particularly rapid bolting, noted in the cms-*nigra* material (Christey 1989). The ATR-cms plants would be useful in hybrid seed production to eliminate pollinators after fertilization (Beversdorf et al. 1985). Also, atrazine resistance will allow planting in areas with atrazine residue.

The horticultural potential of the ATR-cms plants is enhanced by the fact that they appear as vigorous as the parental broccoli material, as shown by field analysis of progeny (in preparation). These ATR-cms plants may be of value in physiological studies on the effect of ATR on vigor. Previous studies with *Brassica* have shown that atrazine resistance has associated yield penalties. For example, the ATR cauliflower developed by Jourdan et al. (1989a) showed lower germination and a slower growth rate, leading to a 3-week delay in maturity as compared to the ATS line (M. H. Dickson, personal communication). In *B. napus,* atrazine resistance is associated with lower biomass and seed yield as compared to an isogenic ATS line (Grant and Beversdorf 1985; Gressel and Ben-Sinai 1985; Röbbelen 1987). Mapplebeck et al. (1982) also noted that seedlings of ATR *B. campestris* were less vigorous than the ATS biotype. In other genera, including *Amaranthus hybridus* (Ahrens and Stoller 1983), *A. retroflexus,* and *Senecio vulgaris* (Conard and Radosevich 1979), atrazine resistance is also associated with reduced vigor, and lower biomass and seed yield. The decreased vigor of these ATR biotypes is thought to be caused by the photosynthetic inefficiency of the ATR

chloroplasts (Radosevich and Holt 1982). However, studies with ATR and ATS biotypes of *Phalaris paradoxa* indicate that atrazine resistance is not always associated with reduced plant vigor (Schonfeld et al. 1987). In *B. napus,* the observation that ATR single-cross hybrids perform better than the ATR parent suggests that conventional breeding could be used to obtain nuclear-cytoplasmic combinations with increased performance (Grant and Beversdorf 1985).

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