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Atrazine-resistant cauliflower obtained by somatic hybridization between *Brassica oleracea* and ATR-*B. napus*

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Summary. Somatic hybridization between *Brassica oleracea* ssp. *botrytis* (cauliflower, 2n=18), carrying the Ogura (R1) male-sterile cytoplasm and *B. napus* (2n=38), carrying a male-fertile, atrazine-resistant (ATR) cytoplasm, yielded three hybrids (2n=56) and six cauliflower cybrids (2n=18), which were selected for resistance to the herbicide in vitro. The hybrids and cybrids were male fertile and self-compatible. They contained both chloroplasts and mitochondria from the ATR cytoplasm. We found no evidence for mtDNA recombination in any of the regenerated plants. Selfed progeny of the *B. oleracea* atrazine-resistant cybrids were evaluated for tolerance to the herbicide in the field. Resistant plants exposed to 0.56–4.48 kg/ha (0.5–4.0 pounds/acre) atrazine in the soil showed no damage at any herbicide level, whereas plants of a susceptible alloplasmic line were severely damaged at the lowest level of herbicide application and killed at all higher levels. These atrazine-resistant cauliflower may have potential horticultural use, especially in fields where atrazine carry over is a serious problem.

Key words: *Brassica oleracea* – Protoplast fusion – Herbicide resistance – Cytoplasmic traits

Introduction

Protoplast fusion permits the combination of unique and desirable cytoplasmic traits not generally attainable by conventional breeding, because in most crop plants the cytoplasm is inherited only from the maternal parent (Fluhr 1983). Unique combinations of cytoplasmic traits may arise by the sorting out of organelles or by the recombination of organelle DNA molecules. Intergenomic recombination of organelle DNA is apparently quite common in the mitochondria of fusion products, but occurs only rarely in the chloroplasts (Pelletier 1986). Our laboratories have been attempting the manipulation of cytoplasmic traits in *Brassica* to obtain agronomically desirable combinations of mitochondria and chloroplasts, as well as to develop procedures that would permit the systematic analysis of organelle genome interaction and inheritance in this genus.

One of our goals has been to obtain many independent fusion products in order to identify possible common patterns of interaction between organelles that would help in the controlled manipulation of specific traits. For these studies we have used two distinct cytoplasms: the Ogura male-sterile cytoplasm (R1) derived from radish (*Raphanus sativus;* Ogura 1968) and the atrazine-resistant cytoplasm (ATR) derived from bird's rape (*Brassica campestris;* Darr et al. 1981). The R1 cytoplasm carries mitochondria that confer cytoplasmic male sterility (cms) and atrazine-sensitive chloroplasts. This cytoplasm has been transferred to cultivated forms of *B. oleracea* and *B. napus,* where it expresses a chlorophyll deficiency at low temperatures (Bannerot et al. 1977). The chlorosis arises from an apparent incompatibility between *Brassica* nuclei and radish chloroplasts (Pelletier 1986). The ATR cytoplasm carries male-fertile mitochondria and atrazine-resistant chloroplasts; it has been available thus far only in cultivars of *B. napus* and *B. campestris* (Ali et al. 1986; Beversdorf et al. 1980).

Another goal of our experiments has been to combine the cms mitochondria from the R1 cytoplasm with herbicide-resistant chloroplasts from the ATR cytoplasm, and to establish this combination within a *B. oleracea* nuclear

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background. The various *B. oleracea* crops are adapted for growth under cool weather conditions. In a typical growing season, varieties carrying the R1 cytoplasm may be exposed to the low temperatures that bring about chlorosis. This chlorosis precludes commerical use of an otherwise highly desirable cytoplasm. Introduction of *Brassica* ATR chloroplasts into R1-*B. oleracea* would eliminate the low temperature-induced chlorosis and, at the same time, provide tolerance to a herbicide that tends to persist over two or more growing seasons in certain types of soils (Gressel 1986). Furthermore, availability of atrazine-tolerant *B. oleracea* would permit the use of this vegetable crop for rotation schemes in fields where atrazine residues are a problem. The replacement of R1 chloroplasts with normal *Brassica* chloroplasts has already been achieved in *B. napus* (Pelletier et al. 1983, Menczel et al. 1987).

We have previously shown that fusion of R1-*B. oleracea* with ATR-*B. campestris* results in somatic hybrids equivalent to the amphidiploid *B. napus* (Robertson et al. 1987; Jourdan et al. 1989). Extensive mtDNA rearrangement was observed in both male-sterile and male-fertile hybrid plants from these experiments, and it remains to be determined if these male-sterile plants exhibit a cms phenotype identical to the one conditioned by the unrearranged R1 mitochodria. In this paper, we report on a fusion between R1-*B. oleracea* (cauliflower; **cc** nucleus) and ATR-*B. napus* (rapeseed; **aacc** nucleus) that resulted in atrazine-resistant somatic hybrids and cybrids. No evidence for mtDNA recombination was obtained in these fusion products.

Materials and methods

Plant material

The cytoplasmic male-sterile *Brassica oleracea* ssp. *botrytis* used in these experiments is a cauliflower inbred line (no. 7642A) developed at the New York State Agricultural Experiment Station, Geneva/NY (Dickson 1985). This line carries the Ogura cms cytoplasm ("R1" as designated by the Crucifer Genetics Cooperative) derived from radish; its main horticultural attribute is a "persistent white" trait that prevents yellowing of the curd in full sun. The atrazine-resistant line is a *B. napus* ssp. *oleifera* cv "Tower" obtained after a series of backcrosses of "Tower" with a wild Bird's rape (*B. campestris*). This weed had developed resistance to the herbicide in corn fields that had been repeatedly sprayed with atrazine (Beversdorf et al. 1980).

Protoplast isolation, fusion and culture

Leaf protoplasts of in vitro-grown cauliflower seedlings were fused with etiolated hypocotyl protoplasts of *B. napus*. The general procedures for protoplast isolation, fusion, and subsequent culture have been described (Jourdan 1988). In the experiment reported here, neither the leaf protoplasts nor the hypocotyl protoplasts were pretreated with iodoacetate or rhodamine 6-G; they were fused by the plate fusion method and, after fusion, protoplasts were cultured only in liquid medium B and C.

Plant regeneration

Colonies obtained after fusion of *B. oleracea* and *B. napus* protoplasts were plated on medium E containing 50 μM atrazine, 1% sucrose and 0.22% Gelrite (Jourdan et al. 1989). Calluses selected for vigor and greening in the presence of atrazine were transferred to fresh medium containing herbicide. After this second transfer, calluses that regenerated shoots were placed on medium F to stimulate shoot development. Calluses that became bleached and grew slowly in the presence of atrazine were transferred to medium E lacking the herbicide. Shoots regenerated from these calluses were then transferred to medium F for continued development. Young plantlets were rooted on MS medium lacking hormones and then transferred to soil.

Characterization of regenerated plants

The procedures for phosphoglucose isomerase isoenzyme characterization, chromosome counts, and organelle DNA characterization were as described (Jourdan 1988; Jourdan et al. 1989). Total cellular DNA was extracted from leaves, digested with different restriction enzymes, electrophoresed, and blotted onto nylon membranes. Cloned fragments of chloroplast and mitochondrial DNA (Palmer and Shields 1984) were a kind gift from Dr. J. Palmer, University of Michigan.

Atrazine resistance

Resistance to atrazine was determined by four methods: (1) measurements of photosynthetic activity in isolated protoplasts with tetrazolium blue (Robertson and Earle 1987); (2) characterization of flash-induced delayed light emission (FIDLE) patterns (Ellenson 1986; J. Ellenson, P. Jourdan and E. Earle, unpublished results); (3) propagation of cauliflower plants on MS medium, lacking hormones but supplemented with 50 μM atrazine, 1% sucrose, 1% agar; (4) application of atrazine to plants in the greenhouse or field (see below).

Progeny studies

All fertile plants were selfed by brushing pollen from the same plant on the stigmas of flowers 1 day after they opened; selfings were continued for as long as flowers were produced. For greenhouse studies, seeds were sown in Cornell mix (Sheldrake and Boodley 1973) in 72-well Speedling trays; seedlings were maintained under continuous illumination provided by cool white fluorescent lamps. To evaluate tolerance to the herbicide, 2-week-old plants were sprayed with the equivalent of 0.56, 1.12, 2.24, and 4.48 kg/ha (0.5, 1.0, 2.0, and 4.0 pounds/acre).

For field studies, seeds were germinated in the greenhouse and transplanted to the field after 5 weeks. Atrazine was applied to the soil just prior to transplant at rates equivalent to the four listed above. The atrazine-susceptible check included in these evaluations was the fertile cauliflower maintainer line for the R1-*B. oleracea* (7642B). Selfed progeny from one plant of 7642B and from one atrazine-resistant cybrid were used in the field evaluation; both the fertile check and the ATR line had the same level of inbreeding. The field studies were carried out in collaboration with Dr. R. Bellinder, Cornell University.

Results

Colony development after fusion

The selection of fusion products was based primarily on the growth inhibition of *B. oleracea* colonies by atrazine

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

Plant	Callus	Chromosome no. (n=)[a]	PGI[b]	Color at 10°C[c]	ATR[d]	mtDNA pattern[e]	Pollen viability[f]	Selfed seed set
B. oleracea		9	S	Y	S	S	–	–
B. napus		19	F	G	R	F	95%	v high
FP-41	10a-1	28	H	G	R	F	65%	low
FP-42	10b-2	28	H	G	R	F	74%	mod
FP-43	10b-2	28	H	G	R	F	85%	mod
FP-44	10b-3	9	S	G	R	F	73%	v low
FP-45	10b-3	9	S	G	R	F	57%	none
FP-46	10b-3	9	S	G	R	F	73%	mod
FP-47	10b-5	9	S	G	R	F	73%	v low
FP-48	10b-5	9	S	G	R	F	61%	none
FP-49	10b-5	9	S	G	R	F	70%	mod
FP-55	10c-3	9	S	Y	S	S	–	–

[a] Chromosome numbers were determined in microsporocytes
[b] PGI – electromorph of phosphoglucose isomerase present in the plants; F – fast, S – slow, H – fast, slow, and intermediate electromorphs
[c] Color was determined in young leaves of plant incubated at 10°C for 2 weeks; Y – yellow, G – green
[d] ATR, response to atrazine, either spray, FIDLE pattern, or tetrazolium assay; S – susceptible, R – resistant
[e] mtDNA pattern: summary of the hybridization pattern of mtDNA; S – characteristic of R1 cms cytoplasm; F – characteristic of ATR, fertile *B. napus*
[f] Pollen viability was determined by acetocarmine staining; approximately 400 pollen grains were evaluated per plant; "–" indicates that no pollen was present

and on the generally poor development that we have observed for *B. napus* hypocotyl protoplasts in medium B. Fusion of cauliflower leaf protoplasts with hypocotyl protoplasts of *B. napus* resulted in the development of 149 colonies that, upon culture on medium containing 50 μ*M* atrazine, gave rise to 38 vigorous, green, putative atrazine-resistant calluses. The remaining colonies grew slowly and were bleached, a typical response of callus susceptible to the herbicide (Grant et al. 1983). Nearly 70% of these bleached calluses recovered when removed from the herbicide-medium; they began to grow more vigorously and many turned green. The 38 putative resistant calluses were cultured for a 2nd month on fresh medium containing the herbicide, but only 16 continued to grow vigorously.

Plant regeneration

Of the 16 calluses selected for vigor after a second passage on atrazine-containing medium, 8 (50%) regenerated one or more plants, whereas only 19 of the 76 calli (25%) whose initial growth was inhibited by the herbicide eventually regenerated plants. These plants were characterized for morphological and biochemical traits to ascertain their nuclear and cytoplasmic composition.

Two types of regenerated plants could be distinguished by leaf texture and overall morphology. One type resembled the *B. napus* parent in overall growth habit and in the presence of trichomes in leaves and stem

Fig. 1. Parents and one of the three hirsute, somatic hybrid plants (FP-42) regenerated from atrazine-resistant callus. *R1 – B. oleracea; A – B. napus*

(Fig. 1); three such hirsute plants were obtained from two calluses selected for vigor on atrazine (Table 1, 10a-1 and 10b-2). The other type of plant resembled the *B. oleracea* parent in growth habit and in having glabrous leaves; 36 such plants were regenerated, 25 from sensitive calluses and 11 from putative herbicide-resistant calluses. Of the 11 glabrous plants derived from apparently resistant callus, 6 plants (regenerated from calluses 10b-3 and 10b-5) could be distinguished from the other 5 (regenerated

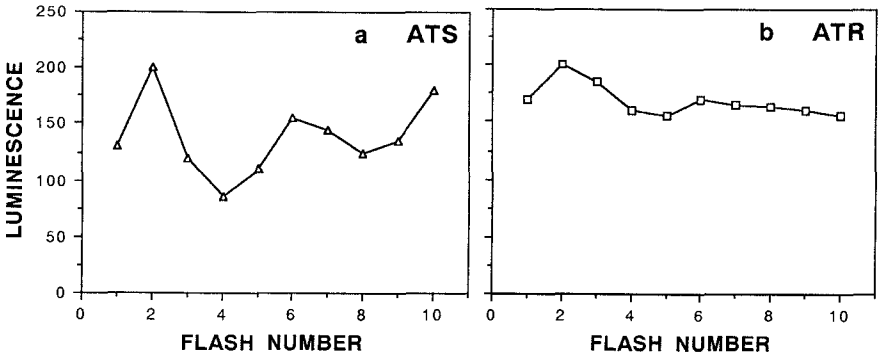

Fig. 2a and b. Flash-induced delayed light emission (FIDLE) patterns characteristic of **a** atrazine-susceptible and **b** atrazine-resistant cauliflower plants. The main feature that distinguishes the two patterns is the more rapidly damped, less-pronounced set of oscillations over the first ten flashes found in the resistant chloroplasts

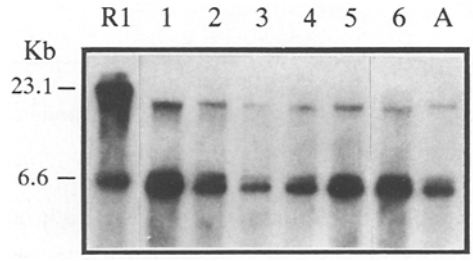

Fig. 3. Chloroplast DNA in atrazine-resistant plants regenerated from fusion. Total leaf DNA was restricted with BglI and probed with a chloroplast-specific DNA fragment from *Petunia*. *R1* – *B. oleracea* (no. 7642A); *lanes 1–6:* FP-41, FP-43, FP-45, FP-46, FP-47, FP-49, respectively; *A* – ATR-*B. napus*. The numbers at left indicate the fragment size in kb

Fig. 4. Flower morphology of parents and cybrid FP-49 (middle). *A* – *B. napus; R1* – *B. oleracea*

from calluses 10c-1-10c-4) by having dark-green leaves. The five light-green plants were similar in color to the 25 plants regenerated from atrazine-sensitive calluses.

Atrazine resistance and low temperature chlorosis in regenerated plants

Characterization of the plants regenerated from putative atrazine-resistant callus was carried out by first evaluating the two chloroplast-associated traits contributed by the fusion partners: resistance to the herbicide (from *B. napus*) and low temperature-induced chlorosis (from *B. oleracea*).

Determination of resistance or susceptibility to atrazine in the regenerated plants was initially done with the tetrazolium blue assay (Robertson and Earle 1987). All 14 plants (3 hirsute and 11 glabrous) regenerated from putative resistant calluses, and 10 of the 25 plants regenerated from sensitive calluses were assayed for photosynthetic activity in the presence of the herbicide. The 10 plants from sensitive callus were sensitive to the herbicide. Of the 14 plants from resistant calluses, only the 3 hirsute plants and the 6 dark-green glabrous plants showed resistance to atrazine by this method (data not shown).

An additional, indirect test for atrazine resistance (FIDLE patterns) was done on some of the regenerated plants. This test measures delayed light emission from chloroplasts after short light pulses (Ellenson 1986); it can detect differences in light emission patterns that occur between atrazine-resistant and wild-type chloroplasts (J. Ellenson, P. Jourdan and E. Earle, unpublished results). The difference in FIDLE patterns arises as a result of the same mutation that confers resistance to the herbicide. Figure 2 shows the typical FIDLE patterns obtained for susceptible and resistant lines. As expected, the three hirsute and six dark-green glabrous plants showed a pattern identical to the atrazine-resistant *B. napus*, whereas the remaining glabrous, lighter green plants showed a wild-type pattern characteristic of atrazine-sensitive *B. oleracea*. Thus, 9 regenerated plants (3 hirsute and 6 glabrous) seemed to contain atrazine-resistant chloroplasts. Further evidence that the 6 dark-green, glabrous, cauliflower-like plants were indeed resistant to the herbicide was provided by in vitro propagation of cuttings from these plants on herbicide-containing MS medium. Cuttings from the putative resistant plants remained green and developed normally in the presence of 50 μM atrazine, while cuttings from sensitive plants died within 2 weeks (data not shown).

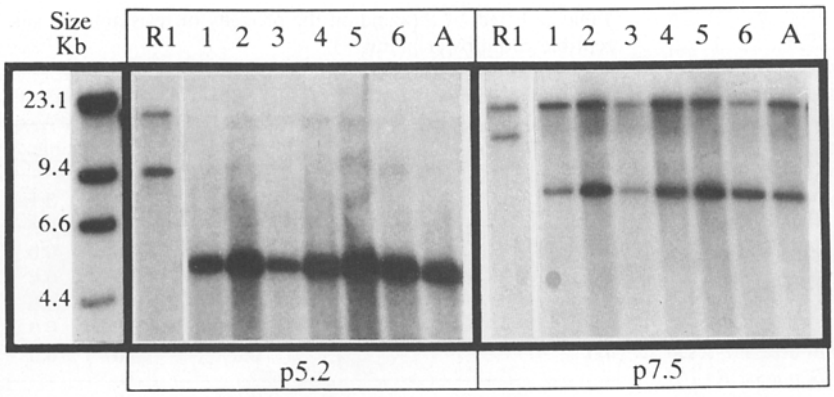

Fig. 5. Mitochondrial DNA in atrazine-resistant, fertile plants. Total leaf DNA was restricted with PstI and probed with p5.2 and p7.5, cloned mitochondrial DNA fragments from *B. campestris* (Palmer and Shields 1984). *R1* – *B. oleracea* (no. 7642A); *lanes 1–6:* FP-42, FP-43, FP-45, FP-46, FP-47, FP-49, respectively; *A* – ATR-*B. napus*. Molecular weight markers at left are derived from HindIII digest of lambda DNA

Assessment of low temperature-induced chlorosis was done by incubating all regenerated plants already in soil at 10 °C for 2 weeks. The 3 hirsute plants and the 6 dark-green, glabrous plants remained green at low temperature; all other glabrous plants turned a pale green or yellow under these conditions.

Final characterization of the chloroplasts in atrazine-resistant plants was done by analysis of chloroplast DNA. Hybridization of filters containing BglI-derived fragments of total cellular DNA with a radiolabelled probe derived from the *Petunia* chloroplast genome (Palmer et al. 1983; Jourdan et al. 1989) revealed that the cpDNA in the 3 hirsute and 6 dark-green glabrous plants was indistinguishable from that of the atrazine-resistant *B. napus* parent (Fig. 3). These analyses confirm that plants regenerated from calluses 10a-1, 10b-2, 10b-3, and 10b-5 all carry atrazine-resistant chloroplasts derived from *B. napus*. Plants regenerated from calluses 10c-1–10c-4; initially selected for growth on 50 μM atrazine, do not contain atrazine-resistant chloroplasts (Table 1).

Fertility of regenerated plants

Characterization of the herbicide resistance trait in regenerated plants was followed by analysis of the male fertility trait associated with mitochondria. The ATR-*B. napus* parent carries normal, fertile mitochondria derived from the wild bird's rape, whereas the R1-*B. oleracea* parent carries cms mitochondria derived from radish.

The atrazine-sensitive glabrous plants flowered within 4–5 months of regeneration. All exhibited the characteristic floral malformations that accompany the *ogu* cms cytoplasm in *B. oleracea* ssp. *botrytis*. These plants probably developed from unfused parental cauliflower protoplasts.

The atrazine-resistant glabrous plants also flowered after about 5 months, but the flowers differed from those of the R1-*B. oleracea* parent both in structure and male fertility (Fig. 4). These flowers had normal petals and stamens that carried anthers full of pollen. The 6 fertile cauliflower plants differed among themselves in ability to set selfed seed; 2 plants (FP-46 and FP-49) yielded relatively good quantities of seed, whereas the others set little or no seed even after repeated pollinations (Table 1).

The 3 hirsute, atrazine-resistant plants flowered within 3 months of regeneration and all had normal flowers that produced large quantities of pollen. The pollen stainability in all the fertile plants was consistently lower than that of the typical fertile *B. napus* (Table 1). Nevertheless, this pollen could be used to self the plants and obtain seeds. The hirsute plants regenerated after fusion produced fewer selfed seed than the *B. napus* parent, but there was seed set in all three (Table 1).

The mtDNA in these 9 atrazine-resistant, fertile plants and in 1 atrazine-sensitive, sterile plant was also analysed. Total cellular DNA was digested with PstI, BglI, HindIII, BstEII, and DraI, and then hybridized with five mtDNA probes derived from the *B. campestris* mitochondrial genome (Jourdan et al. 1989). The 9 atrazine-resistant plants all had mtDNA that appeared to be identical to the *B. napus* parent. We found no evidence for either rearrangement or intergenomic mtDNA recombination between the two parental mitochondrial populations in the fusion products. The atrazine-sensitive plant had mitochondria whose DNA was identical to the cms cauliflower parent. Figure 5 provides an example of the mtDNA hybridization pattern for extracts of 6 resistant plants.

Nuclear composition of atrazine-resistant plants

Characterization of Pgi-2 isoenzymes and chromosome numbers in the 9 atrazine resistant plants revealed that the 3 hirsute plants were somatic hybrids because they contained both the parental (F and S) and the heterodimeric (H) electromorphs of the isozymes (Fig. 6). This pattern is characteristic of plants heterozygous for the locus and is established by the presence of the two parental alleles. In addition, these plants had a haploid set of 28 chromosomes equivalent to the addition of the *B. napus* (n = 19) and *B. oleracea* (n = 9) chromosome sets

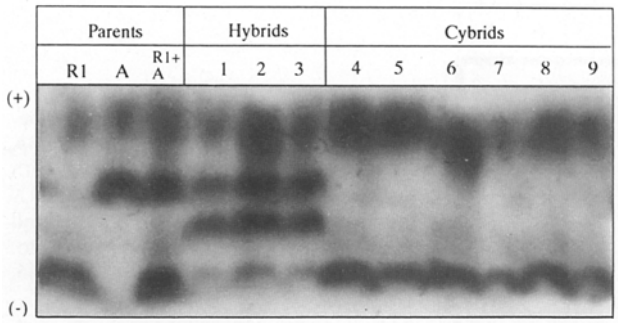

Fig. 6. Phosphoglucose isomerase isoenzymes in atrazine-resistant, fertile plants. *R1 – B. oleracea; A* – ATR-*B. napus; R1 + A* – mixture of *B. oleracea* and ATR-*B. napus* extracts; *lanes 1–3:* FP-41–FP-43; *lanes 4–9:* FP-44–FP-49, respectively

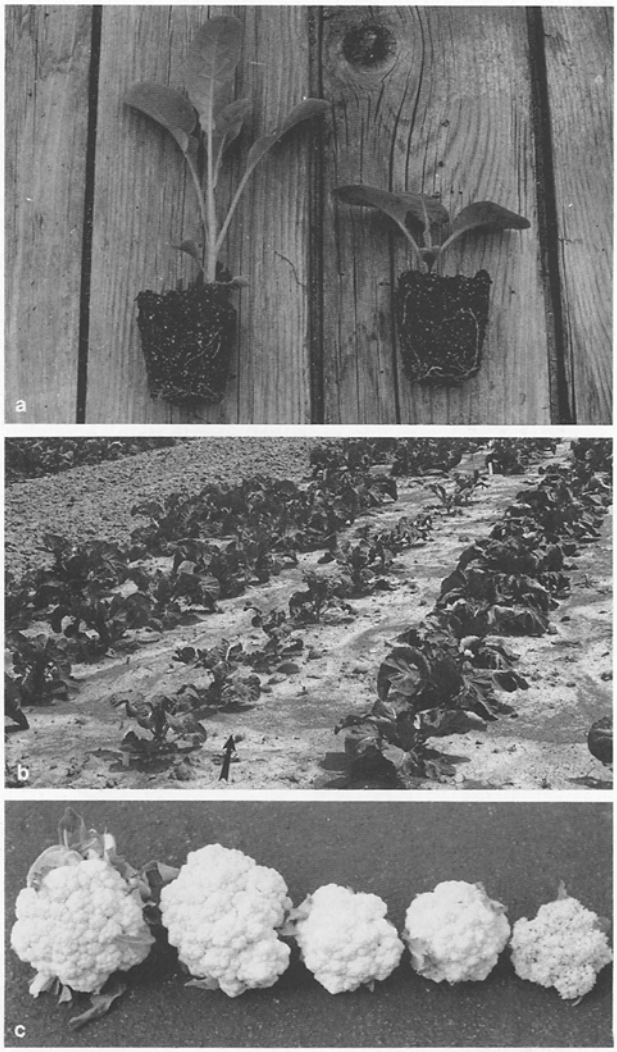

Fig. 7. **a** Growth habit of 4-week-old seedlings from selfed seed of FP-49 (*right:* 7642 Resistant) and the cauliflower maintainer line (*left:* 7642 Susceptible). **b** Contrast in vigor of atrazine-resistant cauliflower plants (*arrow*) and sensitive plants carrying the same nuclear background in fields that were not sprayed with the herbicide. **c** Cauliflower heads produced by atrazine-resistant plants

Table 2. Effect of atrazine on the progeny of resistant and susceptible cauliflower plants[a]

Atrazine treatment (pounds/acre)	Injury rating[b]			
	Resistant		Susceptible	
	Mean	S.E.[c]	Mean	S.E.
0	1.0	0.0	1.0	0.0
0.5	1.0	0.0	3.6	0.6
1.0	1.1	0.1	4.6	0.6
2.0	1.0	0.0	5.0	0.0
4.0	1.0	0.0	5.0	0.0

[a] Atrazine was applied to the soil prior to transplant of cauliflower seedlings

[b] Herbicide-induced injury was based on a scale of 1.0 (no visible injury) to 5.0 (plant killed). Ratings were taken 17 days after transplant and represent the average of four replicate plots for each treatment; 16 plants were usually evaluated per plot (ca. 64 per treatment)

[c] Standard error of the mean

(not shown). A similar characterization of the 6 dark-green, glabrous plants showed that they were cybrids since they contained only the *B. oleracea* Pgi-2 electromorph (S) and had nine haploid chromosomes (Table 1).

Inheritance of the cytoplasmic traits in the hybrids and cybrids

Selfed progeny of the somatic hybrids were evaluated for morphology, fertility and atrazine resistance. Twenty-five progeny plants of FP-41 and FP-42 were grown in the greenhouse. The plants were very uniform in growth habit and flowered within 5 weeks; all were male fertile. Twenty other progeny plants of each hybrid were sprayed with atrazine (2.24 kg/ha) when approximately 2 weeks old; all showed complete resistance to the herbicide.

Selfed progeny of cauliflower cybrid FP-49 were evaluated for resistance to atrazine in the field. These progeny (labelled the R line) were compared to the selfed progeny of a cauliflower plant that carried the same nucleus but in a normal *B. oleracea* cytoplasm (i.e., fertile mitochondria and wild-type chloroplasts, here labelled the S line). Seed germination in the R cybrids was 61% as opposed to >90% in the S line. The R seedlings grew at a much slower rate and had rounder leaves than their S counterparts (Fig. 7). Four-week-old R seedlings had, on average, two leaves with one emerging, whereas S seedlings had four leaves with one emerging by that time. The slower growth rate of R plants was evident throughout the growing season; however, these plants produced fairly normal cauliflower heads (Fig. 7). Evaluation for resistance to atrazine residues in soil was carried out by treating soil with the herbicide to give levels equivalent to 0, 0.5, 1.0, 2.0, and 4.0 pounds per acre. A total of ca. 64 seedlings was planted for each treatment in four replicate

combinations. Table 2 summarizes the ratings for atrazine-induced injury in the R and S plants, 17 days after transplant. None of the 252 R seedlings exposed to atrazine showed any evidence of herbicide injury. Of 310 S seedlings, only the plants in control plots (no atrazine) showed no injury. At the lowest treatment level, plants had serious injury, but some were able to recover after 4–5 weeks; at the higher atrazine levels, all S plants died.

Discussion

The somatic hybridization of R1-*B. oleracea* and ATR-*B. napus* resulted in hybrids ("*B. napoleracea*") and cybrids (*B. oleracea*) which carry both chloroplasts and mitochondria derived from the ATR cytoplasm of *B. napus*. This combination of atrazine-resistant cytoplasm and *B. oleracea* nuclear genome is also being carried out by sexual hybridization via recurrent backcrosses (Ayotte et al. 1987, 1988). However, the cross between these two species is difficult to achieve and, perhaps as a consequence, has not received much attention (Namai et al. 1980; Olsson and Ellerström 1980). Yet many desirable horticultural traits are found in *B. napus* and one of them, resistance to clubroot, has been transferred to *B. oleracea* (Chiang et al. 1977). The most distinct advantage of somatic hybridization is that it has permitted the transfer of cytoplasm from *B. napus* to *B. oleracea* in a single step, accomplished within 6 months, and has allowed field evaluation of the cybrid progenies within 12–18 months.

In addition to producing cybrids with desirable cytoplasmic traits, synthesis of a true hybrid between *B. oleracea* and *B. napus* presents a new type of exotic germplasm that could be evaluated as a rapeseed crop or as a bridge species to transfer traits from *B. napus*. These hybrids (**aaccce**) contain two copies of the diploid *B. oleracea* genome (**cc**) and one copy of the diploid *B. campestris* genome (**aa**). We have preliminary evidence that the somatic hybrids cross quite readily with *B. oleracea*. The somatic hybrids regenerated in this experiment have a similar morphology to the rapeseed *B. napus*. They are self-fertile but produce fewer seed than the *B. napus* parent; this reduced seed set is rather common in various interspecific and intergeneric hybrids of *Brassica* (Namai et al. 1980). Increased seed set is clearly needed before this material can be used as a crop.

Evidence obtained from physiological and biochemical analyses indicated that atrazine resistance in fusion products was derived from the chloroplasts of the atrazine-resistant parent. FIDLE patterns of fusion products exhibited the delayed light emission behavior typical of plants carrying atrazine-resistant plastids. Hybridization of BglI-digested DNA with a chloroplast-specific probe demonstrated the presence of chloroplast sequences derived from *B. campestris*, the original source of the ATR cytoplasm. Thus, the atrazine resistance in these fusion products is not likely to have arisen de novo, as a mutation in culture (Cséplö et al. 1985).

The mitochondria of the fusion products were also inherited from the ATR parent. Normal flower structure, abundant pollen production, self-fertility, and mtDNA hybridization patterns are all characteristics shared by the ATR parent and the resistant hybrids and cybrids. The *B. napus* line used in fusion does not carry nuclear restorers for the R1 cytoplasmic steriliy, so the observed fertility in the three hybrids could not have been due to restoration. The six *B. oleracea* cybrids have the nuclear background of a maintainer for the R1 sterility, again excluding the possibility of nuclear restoration as a determinant of the observed fertility. To our knowledge, there are no reports for the appearance de novo of a restorer gene in plants regenerated from tissue culture.

The lack of mtDNA recombination apparent in the hybrids and cybrids contrasts with the common occurrence of novel mtDNA in other atrazine-resistant hybrids obtained from fusions between R1-*B. oleracea* and ATR-*B. campestris* (Robertson et al. 1987; Jourdan et al. 1989), and between R1-*B. napus* and ATR-*B. napus* (Pelletier et al. 1983; Chetrit et al. 1985). Both Robertson's and Pelletier's groups obtained only one atrazine-resistant hybrid in their experiments, and in each case the mitochondria had undergone apparent recombination. However, only 12 of 19 atrazine-resistant somatic hybrids produced after fusion between R1-*B. oleracea* and ATR-*B. campestris* (Jourdan et al. 1989) showed evidence of possible mitochondrial intergenomic recombination; the remaining 7 had mitochondria from the ATR parent.

The presence of only "fertile" mitochondria in fusion products we selected for atrazine-resistant chloroplasts suggests that the organelles do not sort out at random after fusion of these two cytoplasms. There may be a physiological basis for this result. The ATR cytoplasm originated in a *Brassica* background and, when associated with a *Brassica* nucleus, may have a "competitive" advantage over the R1 cytoplasm, which originated in the more distant *Raphanus* background. Although the R1 cytoplasm is physiologically debilitating to *Brassica* plants, particularly at low temperatures, we have not detected a major deleterious effect of this cytoplasm during the in vitro culture of protoplasts (Jourdan 1988). However, when the two cytoplasms are brought together in fused protoplasts, in vitro conditions may still favor the combination of *Brassica* cytoplasm with *Brassica* nucleus. Since only a small number of fusion products have been obtained thus far, these explanations are obviously speculative.

Our experiments suggest the possibility that the nuclear backgrounds of the fusion partners may influence the behavior of nuclei and organelles in fusion products.

In fusions between *B. oleracea* and *B. campestris,* only hybrids were regenerated and the majority had recombinant mtDNA (Jourdan et al. 1989). In the fusion between *B. oleracea* and *B. napus,* both hybrids and cybrids were obtained and none had recombinant mtDNA. Additional experiments are required to determine if recovery of cybrids after a *napus-oleracea* fusion is a reproducible phenomenon that would permit routine transfer of cytoplasms into *B. oleracea.*

Selection for herbicide resistance in vitro was an effective means of obtaining fusion products with this desired trait. Some sensitive plants were regenerated from putative resistant callus, but the number of plants to be screened for resistance was clearly reduced by the use of atrazine in regeneration medium. Pelletier et al. (1983) screened 85 plants regenerated from 16,000 colonies to find one which carried possible atrazine resistance. No selection for atrazine was done in these experiments. Barsby et al. (1987) also obtained two atrazine-resistant cybrids without specific selection for resistance, but in these fusions, the "donor-recipient" method (Galun and Aviv 1983) was used to eliminate unfused cells. Menczel et al. (1986) used an albino recipient to obtain *Nicotiana* cybrids resistant to another triazine herbicide (terbutryn).

The degree of resistance to atrazine observed in the selfed progeny of one cybrid (FP-49) is equivalent to that found in the *B. napus* parent (Berversdorf et al. 1980). This resistance is extremely high, since atrazine levels eight times greater than those which cause severe damage to susceptible plants show no effect on the resistant plants.

The reduced vigor observed in selfed progeny of the ATR cauliflower is consistent with observations made in other resistant biotypes of various species (Ali et al. 1986; Binding et al. 1982; Conard and Radosevich 1979; Menczel et al. 1985). Studies have suggested that the decreased photosynthetic efficiency of resistant chloroplasts accounts for the reduced vigor of such plants (Gressel 1986). Nevertheless, some of the ATR cauliflower plants produced a horticulturally adequate cauliflower head, in spite of slower growth. Although reduced vigor and lower yields may be a penalty associated with the ATR cytoplasm, it may be possible to increase vigor of ATR plants by proper selection of nuclear genotypes. We have very preliminary indications that F_1 hybrids with the ATR cauliflower cybrids have adequate vigor and, provided that desirable horticultural traits can be maintained, they may be useful plants which exhibit tolerance to residual atrazine in soils. In addition, as Forcella (1987) has shown for *B. napus,* the yield penalty associated with atrazine-resistance is offset by the ability to control weeds in fields with heavy weed infestation.

We would like to voice concern over the widespread development of herbicide-resistant crops. Availability of such crops will have significant beneficial effects only if they are judiciously utilized. Indiscriminate use of herbicides on resistant crops will likely result in the more frequent appearance of resistant weeds, which will only exacerbate an already acute problem (Gressel 1986). Appearance of atrazine-resistant weeds is a premier example of the pitfalls that accompany improper use of a herbicide. In Hungary, where resistant weeds are a serious problem, over 75% of the corn acreage must now be treated with more expensive, and possibly more toxic, herbicides in order to control weeds (Gressel 1986). Such problems can be prevented if proper rotation, cultivation, and general agronomic practices are followed. The availability of atrazine-tolerant *B. oleracea* should not encourage the more intensive use of this herbicide, but should instead provide a measure of flexibility and reliability in soils where residual atrazine can seriously damage an otherwise susceptible crop.

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