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Synthesis of high erucic acid rapeseed (*Brassica napus* L.) somatic hybrids with improved agronomic characters

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Abstract Novel *Brassica napus* somatic hybrids have been created through protoplast fusion of *B. oleracea* var. *botrytis* and *B. rapa* var. *oleifera* genotypes selected for high erucic acid (22:1) content in the seed oil. Fifty amphidiploids (aacc) and one putative hexaploid (aacccc) hybrid were recovered in one fusion experiment. Conversely, only one amphidiploid and numerous regenerates with higher DNA contents were produced in a similar fusion using a different *B. rapa* partner. Hybridity was confirmed by morphology, isozyme expression, flow cytometry, and DNA hybridization. Analysis of organellar DNA revealed a distinct bias toward the inheritance of chloroplasts from the *B. rapa* (aa) genome. All amphidiploids set self-pollinated seed. A erucic acid content as high as 57.4% was found in the seed oil of one regenerated plant. Fatty acid composition was stable in the R_1 generation and was coupled with increased female fertility. Other novel agronomic characters in the hybrids recovered include large seed size, lodging resistance, and non-shattering seed pods.

Key words *Brassica napus* · somatic hybrids · protoplast fusion · Erucic acid

Introduction

Since the first successful re-synthesis of *Brassica napus* by protoplast fusion (Schenck and Robbelen 1982), many other somatic hybrids have been generated. Interspecific and intergeneric hybridization is now possible in the Cruciferae and other plant families where sexual incompatibility barriers once prevented the transfer of valuable genes into agronomically important crops. Since genetic variation is somewhat limited in existing *B. napus* germplasm, protoplast fusion serves to widen the gene pool (Glimelius et al. 1986).

Although protoplast fusion has contributed significantly to the transfer or organellar traits such as cytoplasmic male-sterility and triazine tolerance (e.g., Pelletier et al. 1983; Robertson et al. 1987; Jourdan et al. 1989) and the production of novel *Brassica* vegetable crops (Taguchi and Kameya 1986; Ozminkowski 1992) there has been no attempt to alter the fatty acid composition of *B. napus* through fusion of a specifically selected parental germplasm. Rapeseed re-synthesized through sexual crosses between *B. alboglabra* and *B. rapa* showed some interesting changes in fatty acid composition, such as a transgressively high oleic acid (18:1) content (Chen and Gertsson 1988; Chen and Heneen 1989). This suggests that protoplast fusion may be a valuable route for the manipulation of fatty acid composition.

In addition to its use for altering fatty acids in canola-type *B. napus* cultivars grown for human consumption, protoplast fusion may also help produce lines with increased levels of erucic acid (22:1), a long-chain fatty acid with numerous industrial applications including its potential industrial use as a lubricant, slip agent, fuel and fuel extender, surfactant, engineering thermoplastics, coating, adhesive, agrichemical and resin (Pryde and Rothfus 1989). In the 1960s, it was determined that the 22:1 level of the seed oil was governed by two loci, each with several alleles, in *B. napus* (Harvey and Downey 1964), and by a single locus with two alleles in *B. rapa* (Dorrell and Downey 1964). This hypothesis was later substantiated by Chen and Heneen (1989), who also found three out of four sexually re-synthesized hybrids, showed partial epistasis for a high 22:1 level.

Rapid recovery of a potentially diverse array of fusion products, together with increased germplasm utilization, make protoplast fusion an attractive system for targeted fatty acid modifications in rapeseed. Improvements in oil content, early flowering and maturity, lodging and shattering resistance, tolerance to insects,

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diseases or temperature extremes, and increased geographic adaptation through altered photoperiodic response, may also be possible via protoplast fusion.

This paper describes the fatty acid composition of *B. napus* somatic hybrids produced by protoplast fusion of *B. oleracea* and *B. rapa* accessions selected for high levels of erucic acid. Other characteristics of these plants and their progeny are also assessed.

Materials and methods

Parental germplasm selection

One accession of *B. oleracea* var. *botrytis* (PI 372860, a dwarf cauliflower with 62.9% 22:1), and three accessions of *B. rapa* (fusion 1 = PI 268371, var. *rapifera*, 54.3% 22:1; fusion 2 = PI 370729, var. *oleifera*, 53.2% 22:1; fusion 3 = PI 347609, var. *oleifera*, 53.4% 22:1) were used in the protoplast fusion experiments. These PIs were selected from more than 2100 accessions of *Brassica* spp. from 23 germplasm collections worldwide previously screened for fatty acid composition (Auld et al. 1988; Mahler and Auld 1989) because they were reported to have the highest levels of high 22:1 (Table 1). The *B. oleracea* accession also showed higher shoot regeneration from protoplasts than other comparable lines tested (Heather 1993). The *B. rapa* accessions selected were all self-compatible and morphologically diverse (Heather 1993). They were recalcitrant in protoplast culture, which simplified the pre-treatment and hybrid selection strategy.

Protoplast isolation, fusion and plant regeneration

Leaf mesophyll protoplasts were isolated from 100–200-mg pieces of the youngest expanded leaves from *B. oleracea* and *B. rapa* plants germinated and grown in vitro essentially as described by Robertson and Earle (1986). Protoplasts were fused as described by Menczel and Wolfe (1984) and Thomzik and Hain (1988) with minor modifications. *B. oleracea* protoplasts were treated with 5 mM iodoacetate (IA) to prevent division and improve the efficiency of hybrid selection. The final density of intact protoplasts plated ranged from 2.8×10^4/ml for fusion 2 to 9.5×10^4/ml for fusion 3. The feeder layer system described by Walters and Earle (1990) was used for the first 20–30 days of culture. Shoots were regenerated on medium E of Pelletier et al. (1983), with BAP instead of IPA and no NAA. All shoots were rooted on MS (Murashige and Skoog 1962) medium with 3% sucrose and no growth regulators. Rooted plants were acclimated slowly to air after potting in Cornell mix (Sheldrake and Boodley 1973) and grown to maturity in a greenhouse.

Hybrid verification

Hybrids were verfied by morphological observations, flow-cytometric estimation of nuclear DNA content (Arumuganathan et al. 1991), and isozyme analysis using a cellulose acetate electrophoresis system (Heather 1993). Phosphoglucoisomerase (PGI) produced a clearer banding pattern than phosphoglucomutase (PGM). Hybridity was further confirmed through DNA hybridization.

DNA studies

Total DNA was isolated from young, healthy leaf tissue essentially according to the urea-based miniprep reported by Shure et al. (1983), digested with *Eco*RI, and electrophoresed in 0.8% agarose. Transfer of DNA to nylon membranes, pre-hybridization, hybridization and washing followed the procedure of Church and Gilbert (1984). Studies of hybridity at the nuclear level used probe AC11B6 (kindly provided by Dr. T. C. Osborn, University of Wisconsin-Madison), a cDNA clone derived from flower-bud mRNA of flowering pak choi. It hybridizes to different fragments in each diploid parent. A chloroplast DNA (cpDNA) probe (8a), which is a 3.8-kb *Pst*I-*Sal*I fragment from chloroplasts of *Oncidium excavatum*, was selected for its ability to differentiate the plastid genomes of *B. oleracea* and *B. rapa*. It was provided by Dr. J. Palmer, Indiana University, Bloomington, Indiana, via Dr. T. C. Osborn.

Fertility and fatty acid analysis

Pollen stainability (% healthy grains) was determined by squashing anthers 0–1 days after anthesis in a drop of acetocarmine and counting several fields of 200 pollen grains under a microscope at 160x. Pollen grains that were plump, round, and stained red are generally regarded as viable while those that were shrivelled and did not stain are considered not viable. Flowers were both bud and open-flower pollinated, and the mean ± SE number of seeds per pod was calculated based on 20 randomly selected pods. Fatty acid analyses were done on 100-mg seed samples as described by Mahler and Auld (1989). Oil content was determined from 2.5-g random samples of seed by nuclear magnetic resonance (NMR) using a sealed standard containing 43.0% oil.

Field trials

During the summers of 1991 and 1992, vernalized (4 weeks at 5 °C) and non-vernalized 6 week-old seed-grown plants were transplanted in a field site located in Ithaca, N.Y. Plants were set on May 10, 1991 and May 15, 1992 in a randomized complete block with four replications. Plants were both open pollinated (OP) and manually selfed. Fully dried seed was harvested by hand.

Results

Plant regeneration

A total of 512 calli was recovered from the three fusions. Development of calli from plated protoplasts is illustrated in Fig. 1 a. The fusion-derived calli (arrow) often grew more rapidly than calli from unfused parental protoplasts. In fusion 1, no shoots were regenerated despite the recovery of 132 calli. In fusions 2 and 3, 13% of the calli produced at least one shoot (Table 1). Shoots were recovered 8–12 weeks after plating. From these shoots, 109 plants were grown to maturity.

Morphology and hybrid verification

Leaf morphology of 50 out of 51 somatic hybrids from fusion 2 resembled typical rapeseed cultivars with numerous lobes along the base of the petiole (Fig. 1 b). However, many regenerates from fusion 3 exhibited crinkled, thicker leaves with a somewhat brittle texture (data not shown). These plants were hexaploids (see below).

Isozyme patterns for phosphoglucoisomerase (PGI) indicated that all 109 regenerated plants from fusions 2 and 3 were somatic hybrids (Fig. 2). The putative hybrids exhibited both parental bands plus a heterodimeric intermediate band (arrow) at the *Pgi-2* locus, as described by Arus and Orton (1983).

Flow-cytometric analysis of isolated nuclei confirmed the hybrid nature of the regenerated plants but

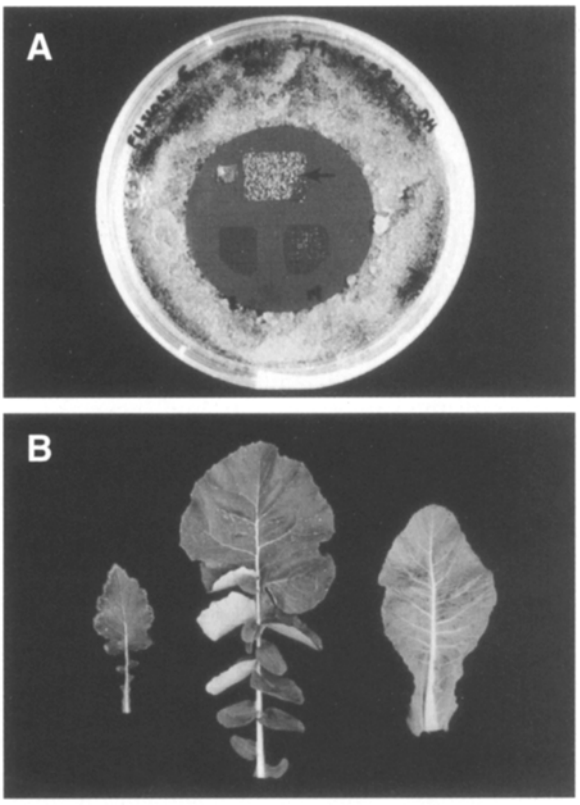

Fig. 1 **A** Culture system with 15 day-old protoplast-derived calli growing on the filter over the feeder layer. Fast-growing fusion calli are indicated by the *arrow* (upper portion of filter). The lower portion contains IA-treated *B. oleracea* protoplasts which produced no calli (left) and calli from *B. rapa* protoplasts (right). **B** Fully-expanded leaves (left to right) of *B. rapa* parent, somatic hybrid, and *B. oleracea* parent from fusion 2

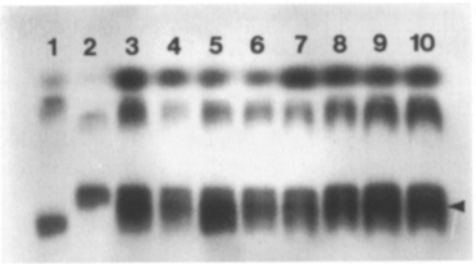

Fig. 2 Typical phosphoglucoisomerase (PGI) banding pattern for fusion 2. *B. oleracea* parent (*lane 1*), *B. rapa* parent (*lane 2*), and somatic hybrids (*lanes 3–10*) exhibiting both parental bands plus a heterodimeric intermediate band (*arrow*)

also indicated higher DNA contents for some of them (Table 1). Measurements of unfused protoplasts of the fusion partners gave values of 1.10 ± 0.01 pg. for *B. rapa* (aa) and 1.24 ± 0.02 pg for *B. oleracea* (cc). In fusion 2, all plants except one were normal amphidiploids with an average 2 C value of 2.29 pg. Conversely, fusion 3 produced only one confirmed amphidiploid (2.32 pg); the remaining 57 plants contained significantly more DNA (Table 1). Forty-four of these were probably aacccc hexaploids from fusions between one *B. rapa* protoplast and two *B. oleracea* protoplasts. Such fusions would give a 2 C value of 3.58 pg (1.10 + 2 × 1.24), very close to the observed mean of 3.56 pg. The six putative aaaacc regenerates had a mean DNA content of 3.44 pg, exactly the numerical addition of two *B. rapa* and one *B. oleracea* protoplasts. Figure 3 shows a histogram generated from the data of fusion 3.

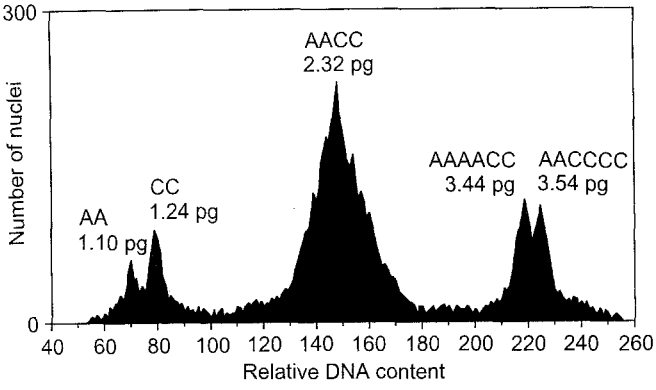

Fig. 3 Flow-cytometric-generated histogram from fusion 3 showing peaks: *AA* = *B. rapa* parent, *CC* = *B. oleracea* parent, *AACC* = amphidiploid regenerate, and putative hexaploids *AAAACC* and *AACCCC*. The histogram is a composite of five samples deliberately mixed together

Table 1 Regeneration and plant evaluation of *B. napus* somatic hybrids from three fusion experiments

Fusion number	Calli[a]	Plants[b]	Isozyme expression[c]	DNA content[d]	Interpretation
1	132 (0)	–			
2	167 (21)	50	O + R	2.29 ± 0.03	Resynthesized *B. napus*
		1	O + R	3.51	*B. napus* plus extra *oleracea*
3	213 (27)	1	O + R	2.32	Resynthesized *B. napus*
		44	O + R	3.56 ± 0.05	*B. napus* plus extra *oleracea*
		6	O + R	3.44 ± 0.02	*B. napus* plus extra *rapa*
		7	O + R	4.09 ± 0.10	*B. napus* plus extra DNA

[a] Number of calli on regeneration medium and the number producing at least one shoot in parentheses
[b] Number of plants regenerated and grown to maturity
[c] Isozyme banding pattern for phosphoglucoisomerase (PGI). *B. oleracea* is designated by the letter O and *B. rapa* by R
[d] Nuclear DNA content (2 C value in picograms ± SE) as determined by flow cytometry

Hybridization of *Eco*R1-digested DNA with the nuclear probe AC11B6 provided definitive evidence that all fusion products were somatic hybrids. Four fragments of approximately 1.8 and 3.1 kb (*B. oleracea* parent) and approximately 2.6 and 4.0 kb (*B. rapa* parent) were observed for all putative hybrids tested (Fig. 4a).

Chloroplast DNA analysis

Chloroplast inheritance was variable, but showed a distinct bias toward the aa genome. The probe 8a hybridized to a fragment of approximately 7.9 kb in the *B. oleracea* fusion partner and a band of about 5.5 kb in the *B. rapa* partner (Fig. 4 b, c). The single amphidiploid regenerate from fusion 3 displayed a *B. oleracea* cpDNA pattern [lanes 1 (R0) and 2 (R1), Fig. 4 b], but only a few of the amphidiploids from fusion 2 contained *B. oleracea* cpDNA (lane 4, Fig. 4 b; lanes 6 and 15, Fig. 4 c). Of 41 putative amphidiploids from fusion 2 analysed, 83% showed *B. rapa* cpDNA banding patterns while 14.6% showed a *B. oleracea* cpDNA pattern. Other similar fusions resulted in up to 100% *B. rapa* chloroplast inheritance (Heather 1993). A combined pattern (aa + cc) was exhibited by the sole putative aacccc plant from fusion 2 which accounts for the remaining 2.4%. In fusion 3, 79% of the regenerates (putative aacccc) exhibited the same combined chloroplast pattern. Amphidiploid progeny (aacc) were obtained by crossing a putative hexaploid (aacccc) with line 15 (Quazi, 1988) as the pollen parent. The progeny contained only the 5.5-kb (aa) fragment (data not shown). Loss of the 7.9-kb *B. oleracea* band initially present thus accompanied loss of the extra cc genome.

Pollen stainability and seed set

Pollen stainability varied widely both between fusions and between normal amphidiploids and those hybrids with higher ploidy within fusions. Fusion 2 amphidiploids had a significantly higher grand mean (96% stained) than the single amphidiploid regenerate from fusion 3 (49% stained). Putative hexaploids had significantly lower pollen stainabilities than their amphidiploid counterparts for fusions 2 and 3 (33% and 26% stained, respectively).

Self-pollinated seed set for the fusions showed a strong correlation with pollen stainability. After bud pollination, R_0 plants from fusion 2 produced three- to four-times as much self seed (mean = 4–5 seed/pod) than those from fusion 3 (mean = 1.3 seeds/pod). Pollination of open flowers from fusion 2 amphidiploids resulted in 2–4 seeds/pod, but no seed from the single fusion 3 amphidiploid. No self-pollinated seed was recovered from putative hexaploid plants. The field open-pollinated and selfed seed yield of fusion 2 somatic hybrids increased significantly to 24.6 ± 3.1 seeds/pod in the R_1 generation.

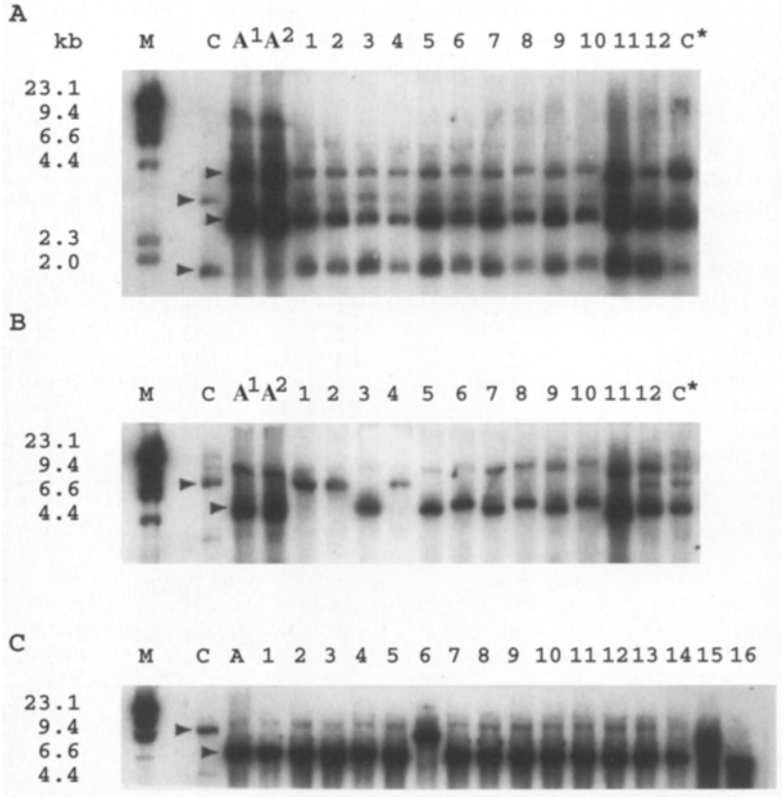

Fig. 4 Hybridizations of AC11B6 nuclear (**A**), and 8a chloroplast (**B**) probes to *Eco*RI digests of parents and somatic hybrids from fusions 2 and 3. *Lane M* is Lambda DNA cut with *Hin*dIII, *C* is the *B. oleracea* parent, A^1 and A^2 are the *B. rapa* parents of fusions 3 and 2, respectively. *Lanes 1 and 2:* R_0 and R_1 of the fusion-3 amphidiploid 3–3; *lane 3*: fusion-3 putative hexaploid (aaaacc) plant 3–6; *lanes 4–11:* amphidiploids from fusion 2; *lane 12:* fusion-2 putative hexaploid (aacccc) plant 2–5; *lane C**: mixture of parental lines (aa + cc). **C** Hybridization of 8a chloroplast probe to *Eco*RI digest of parents and additional amphidiploids from fusion 2 (*lanes 1–16*). *Arrows* indicate specific fragments of interest

Fatty acid analysis

As much as 57.4% erucic acid (22:1) was present in the seed oil of several self-pollinated R_0 somatic hybrids from fusion 2 (Table 2). This level was found to be stable in field-grown R_1 progeny of the best R_0 selection (sample 2–9), with a 22:1 level of 56.6%. This result was particularly encouraging because the 22:1 levels of the parental lines grown under the same conditions decreased in comparison to the originally tested values. This decrease was most striking for the *B. oleracea* fusion partner, which dropped from 62.9% to 43.3% 22:1 (Table 2). It was noted that when 22:1 values decreased from previously recorded levels, oleic, linoleic and linolenic acids increased. Most of the somatic hybrids generated had 22:1 levels near the mid-parental mean (about 45%) of parents selfed at the same time as the hybrids (Heather 1993). Mid-parental means were also generally found for all other fatty acids from fusion-2 seeds (Table 2). The single fusion-3 hybrid (aacc) did not follow this trend, however. It had transgressively lower 22:1 and higher palmitic and stearic acids than the fusion-2 hybrids.

Oil content of the fusion product highest in 22:1 (2–9) was 29.8% (near the mid-parental mean of 29.1%). The content was slightly higher (31.4%) in the R_1 from a bulked field-grown sample of this line.

Novel agronomic characteristics in somatic hybrids

Plants from fusions 2 and 3 flowered as late annuals, with spring-planted R_1 plants not flowering until late August at the East Ithaca, N. Y., site. It was possible to accelerate the time until first flower by giving 4–6 week-old plants a vernalization period of 4 weeks at 5 °C. Therefore, this line could be grown as either a late-season annual or a biennial in relatively mild temperate zones.

Three agronomically desirable traits were observed in the majority of somatic hybrids generated from fusion 2. First, short main-stem length was inherited (Fig. 5a), presumably from the dwarf cauliflower parent. This is an attractive trait which has provided some resistance to lodging in preliminary field tests. Axillary shoots often pushed out during the rosette-stage, as in the cauliflower accession, which often lacked a central terminal curd. Secondly, large seed size (up to 3.0 mm diameter) was inherited from the *B. rapa* parent. The seed is about twice the diameter of 'Westar' *B. napus*, as illustrated in Fig. 5 b. Finally, all somatic hybrids from fusion 2 had non-shattering seed pods. Even when fully dry in the field, no shattering of pods was observed in four separate replicated rows of each R_1 line while shattering was observed for two *B. napus* controls 'Bridger' and 'Cascade'. In addition, several somatic hybrids showed good

Table 2 Fatty acid (FA) composition of original parental accessions and selfed seed of parents and somatic hybrids from fusion experiments 2 and 3. For fusion 2 only the ten selections with the highest 22:1% are listed

Sample ID	Fatty acid composition[a] (% methyl ester)						
	16:0	18:0	18:1	18:2	18:3	20:0	**22:1**
Reported FA composition of parental seed[b]							
B. oleracea	4.8	0.0	7.9	13.5	7.1	3.8	**62.2**
B. rapa (fusion 2)	2.1	1.0	14.8	12.1	9.1	5.7	**53.2**
B. rapa (fusion 3)	1.7	1.1	18.5	13.6	5.1	6.6	**53.4**
Parental and somatic hybrid FA compositions after selfing in greenhouse[c]							
B. oleracea	5.9	0.6	14.0	20.4	11.3	4.5	**43.3**
B. rapa (fusion 2)	1.5	1.6	25.9	9.8	4.9	7.6	**48.7**
B. rapa (fusion 3)	1.9	1.1	17.0	12.9	6.9	5.0	**55.1**
2–9	2.2	1.0	14.1	13.7	7.4	4.2	**57.4**
2–4	2.3	1.1	16.0	14.7	6.5	4.2	**55.2**
2–1	2.7	1.2	17.1	14.7	6.1	4.7	**53.5**
2–24	2.5	1.1	18.3	15.1	4.4	5.7	**52.2**
2–47	2.6	1.1	17.8	15.9	4.5	5.7	**52.2**
2–40	2.5	1.3	19.1	15.2	4.2	5.5	**52.0**
2–7	2.9	1.1	16.4	15.0	7.2	4.5	**51.8**
2–35	2.6	1.0	18.4	15.9	4.8	5.1	**51.8**
2–17	2.6	1.2	19.3	15.8	5.1	4.9	**50.8**
2–36	3.5	0.9	19.7	16.0	3.9	6.5	**48.8**
LSD (5%)[d]	NS	NS	7.8	NS	2.9	NS	4.6
3–3	5.2	3.7	21.9	15.1	5.5	7.6	**41.0**

[a] Percent of total fatty acids. Data are the means of four randomly selected 100-mg seed replications for: 16:0 = palmitic, 18:0 = stearic, 18:1 = oleic, 18:2 = linoleic, 18:3 = linolenic, 20:1 = eicosenoic and 22:1 = erucic acids. The top ten fusion-2 hybrids are shown ranked by highest percent 22:1. Values for the other 35 hybrids analyzed are listed by Heather (1993)

[b] Original FA analysis done at the Univ. of Idaho on seed from PI stations at Pullman, Wash. (*B. oleracea* stocks) and Ames, Iowa (*B. rapa* stocks). The *B. oleracea* parent for both fusions was PI 372860. The *B. rapa* parent in fusion 2 was PI 370729 and in fusion 3 was PI 347609

[c] All samples selfed simultaneously under mean maturation temperatures of 28.6 ± 2.5 °C day and 21.5 ± 0.7 °C night

[d] Protected least significant difference (LSD) based on all 50 regenerates from fusion 2

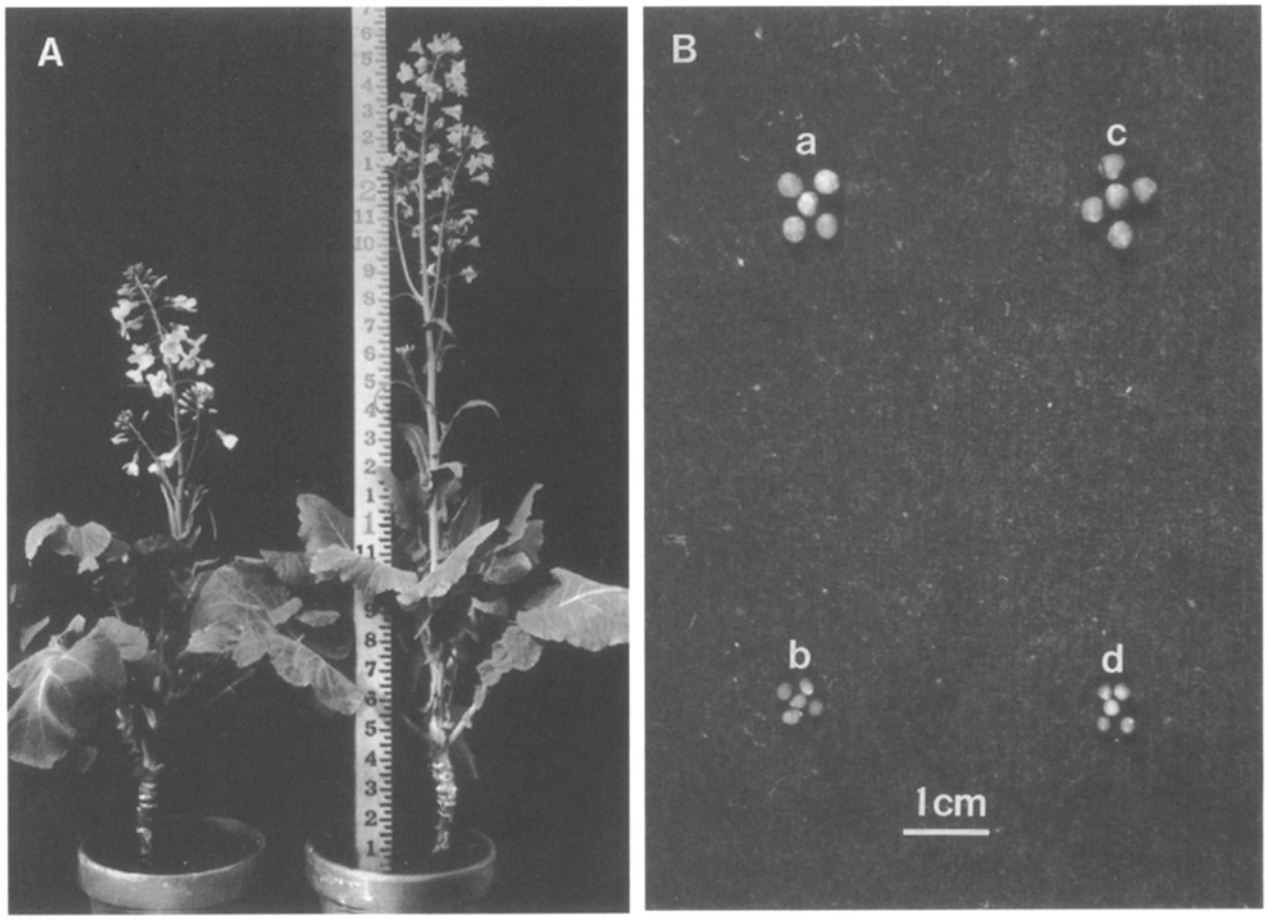

Fig. 5 **A** Contrasting plant height during the same stage of flowering for *B. napus* regenerates from fusion 2 (left) and another *B. oleracea* + *B. rapa* fusion (Heather 1993) (right). **B** Seed size distribution for selfed seed of fusion 2: *a B. rapa* parent (2.5–2.8 mm), *b B. oleracea* parent (1.4–1.6 mm diameter), *c* typical somatic hybrid (2.5–3.0 mm), and *d B. napus* cv Westar (1.4–1.6 mm)

tolerance to powdery and downy mildew while 'Bridger' and 'Cascade' were highly susceptible when grown in contaminated growth chambers (data not shown).

Discussion

A large and diverse array of somatic hybrids was produced in this study, including several agronomically interesting lines that show promise for future breeding. Pre-treatment of *B. oleracea* protoplasts, coupled with poor regeneration of *B. rapa* protoplasts, was an efficient stategy for the selection of hybrids, as reported for similar fusions (Jourdan et al. 1989; Osminkowski 1992). No escapes of the *B. oleracea* parent were observed for fusions 2 and 3 or for 5 out of 7 other similar fusion experiments that produced plants (Heather 1993). It is intriguing that no shoots were regenerated from fusion 1, which had the same *B. oleracea* parent as fusions 2 and 3. Interestingly, the *B. rapa* parent in fusion 1 was the only accession out of 15 tested that regenerated a shoot from unfused *B. rapa* protoplasts (Heather 1993).

Morphology, isozyme expression, flow-cytometric analyses and DNA hybridization patterns collectively provided solid confirmation that the plants recovered were somatic hybrids. Flow cytometry was rapid and able to provide consistent estimations of nuclear DNA content that were easily interpreted in terms of ploidy (Table 1 and Fig. 3). This precision is desirable from a breeding standpoint, as regenerates with higher ploidy can either be quickly discarded or the next appropriate breeding step can be determined. For example, self-pollinated seed could not be obtained from putative hexaploid regenerates. However, semi-synthetic crosses of aacccc plants with *B. rapa* (aa) as the pollen parent were successful. It was also possible to cross these plants with line 15 (Quazi 1988) as the pollen parent.

Based on flow-cytometric results, the majority of the fusion-3 regenerates appeared to be hexaploids (aacccc), resulting probably from the fusion of two *B. oleracea* protoplasts with one from *B. rapa*, as reported by others (Sundberg et al. 1987; Terada et al. 1987). Sundberg et al. (1987) also described hybrids with 50–60 chromosomes that exhibited convoluted leaf morphologies similar to that of the fusion-3 plants. It is possible that non-genic characters of the nuclear DNA are affecting the phenotype independent of encoded information. Such "nucleotypic" effects due to nuclear size have been discussed by Bennett (1971).

Several recent reports have discussed cytoplasmic inheritance in somatic hybrids of *B. napus* (Sundberg et al. 1987; Sundberg and Glimelius 1991). The analysis of chloroplast genomes in the present study suggests a strong bias toward inheritance from the aa genome. Pre-treatment of *B. oleracea* protoplasts with the metabolic inhibitor iodoacetate may have contributed to this bias, as suggested by Siderov et al. (1981). *B. rapa* plastids may dominate after several cycles of cell division because they were not metabolically impaired and their associated cytoplasm permits cell division via complementation. Thus, a higher ratio of *B. rapa* : *B. oleracea* plastids may exist after initial divisions, resulting in bias toward aa genome chloroplasts during sorting out. A bias for *B. rapa* chloroplasts has been observed in similar fusions in another laboratory (Dr. P. Jourdan, personal communication).

Some of the hybrids generated in the present study exhibited very high pollen stainability and moderate to good seed set in the R_0, as did some hybrids reported by Jourdan et al. (1989). Open-pollinated seed set for R_1 progeny of fusion 2 line 2–9 approached a commercially-acceptable level (24.6 ± 3.1 seeds per pod). Other hybrids, both of normal and higher ploidy, had relatively low pollen stainability and seed set, similar to the results of Sundberg et al. (1987). Choice of parental genotypes appears to be an important factor. Fusions 2 and 3 differed only in the *B. rapa* parent. Although both were oilseed types, pollen stainability and seed set were significantly higher for fusion 2. However, this may also be due to aspects of the fusion process itself (number of protoplasts fused and subsequent ploidy) rather than genotypic effects.

Fusion 2 produced promising high 22:1 breeding lines (line 2–9 being selected for highest 22:1). Initial field trials of R_1 progeny of line 2–9 showed that the high ($> 55\%$) content of 22:1 was stable. Lower 22:1 levels prevailed in selfed progeny of the *B. oleracea* parent, suggesting that the original reported value was higher than the true average. In a previous study, it was demonstrated that the amount of 22:1 in the seed oil could effectively be cut in half by maturing the plants at high temperatures (Heather 1993). It is possible that the original *B. oleracea* seed lot tested was matured under cool air temperature, thus increasing the amount of 22:1. Additionally, the original fatty acid analyses were done on single 500-mg samples rather than on several replicated random samples. Finally, the PIs were open pollinated, increasing the chances of heterogeneity in the entire population. In any case, we recovered a somatic hybrid line with a transgressively higher 22:1 that proved stable though several generations and a range of maturation temperatures (field bulks varied from those obtained in the greenhouse). The present study has shown that it is possible not only to enhance genetic variability through protoplast fusion, but also to successfully select for, and fix, desirable characters from original fusion products.

Resistance to lodging, shattering, and powdery mildew are other desirable characters present in these somatic hybrids. Genetically inherent lodging resistance seems an economically and environmentally desirable alternative to the application of synthetic growth regulators (Armstrong and Nicol 1991). The large seed size (up to 3.0 mm diameter) may provide better seedling emergence through crusted soil (Heather and Sieczka 1991) and resistance to flea beetles at the vulnerable seedling stage (Bodnaryk and Lamb 1991). Because this line can be grown both as a late annual or a biennial in temperate regions with relatively mild winters, it shows promise for use in varied geographic locations. Preliminary field trial results at Ithaca, N. Y., indicated good overwintering in our area during normal winters.

We have demonstrated that the production of *B. napus* somatic hybrids by protoplast fusion can be a powerful breeding tool to generate variability. Traditional rapeseed breeding has been limited to genetic exchange between *B. rapa* and *B. napus*. Through protoplast fusion, it may be possible to expand the *B. napus* gene pool by introgressing agronomically valuable genes from *B. oleracea*.

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