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Production of intertribal somatic hybrids between *Brassica napus* L. and *Lesquerella fendleri* (Gray) Wats

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Abstract Intertribal *Brassica napus* (+) *Lesquerella fendleri* hybrids have been produced by polyethylene glycol-induced fusions of *B. napus* hypocotyl and *L. fendleri* mesophyll protoplasts. Two series of experiments were performed. In the first, symmetric fusion experiments, protoplasts from the two materials were fused without any pretreatments. In the second, asymmetric fusion experiments, X-ray irradiation at doses of 180 and 200 Gy were used to limit the transfer of the *L. fendleri* genome to the hybrids. X-ray irradiation of *L. fendleri* mesophyll protoplasts did not suppress the proliferation rate and callus formation of the fusion products but did significantly decrease growth and differentiation of non-fused *L. fendleri* protoplasts. In total, 128 regenerated plants were identified as intertribal somatic hybrids on the basis of morphological criteria. Nuclear DNA analysis performed on 80 plants, using species-specific sequences, demonstrated that 33 plants from the symmetric fusions and 43 plants from the asymmetric fusions were hybrids. Chloroplast and mitochondrial DNA analysis revealed a biased segregation that favoured *B. napus* organelles in the hybrids from the symmetric fusion experiments. The bias was even stronger in the hybrids from the asymmetric fusion experiments where no hybrids with *L. fendleri* organelles were found. X-ray irradiation of *L. fendleri* protoplasts increased the possibility of obtaining mature somatic hybrid plants with improved fertility. Five plants from the symmetric and 24 plants from the asymmetric fusion experiments were established in the greenhouse. From the symmetric fusions 2 plants could be fertilised and set seeds after cross-pollination with *B. napus*. From the asymmetric fusions 9 plants could be selfed as well as fertilised when backcrossed with *B. napus*. Chromosome analysis was performed on all of the plants but 1 that were transferred to the greenhouse. Three plants from the symmetric fusions contained 50 chromosomes, which corresponded to the sum of the parental genomes. From the asymmetric fusions, 11 hybrids contained 38 chromosomes. Among the other asymmetric hybrids, plants with 50 chromosomes and with chromosome numbers higher than the sum of the parental chromosomes were found. When different root squashes of the same plant were analysed, a total of 6 plants were found that had different chromosome numbers.

Key words *Brassica napus* · Fertile intertribal somatic hybrids · *Lesquerella fendleri* · Organelle segregation · X-ray irradiation

Introduction

Brassica napus is one of the world's most important oil crops and is well-adapted to regions with a temperate climate. Oil as well as the seed meal and verdure of *B. napus* can be utilised for different nutritional and industrial objectives. Even though the fatty acids of *B. napus* oil are suitable for many uses, including consumption, an attractive goal for *B. napus* breeders is to modify the fatty acid composition to make the oil more suitable for industrial purposes. In the Brassicaceae family several wild species have a vast diversity of fatty acids in their storage lipids. *Lesquerella fendleri*, which belongs to the tribe *Drabae* of the Brassicaceae, is one of the chief candidates for plant breeding purposes and domestication (Muuse et al. 1992), given that lesquerolic oil has a high content of the economically important hydroxy fatty acids, and defatted *L. fendleri* meal contains several valuable amino acids. Moreover, since *L. fendleri* can grow in areas with pure soils and tolerates drought and low temperatures (Gentry and Barclay 1962), it has

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potential value as a gene donor for the modification of *B. napus*.

Potentially useful agronomic traits from wild species can be introgressed into the genome of cultivated crops by hybridisation or transformation. With transformation alien DNA can be integrated directly into the target nuclear DNA, thereby excluding the problems of genetic incompatibility (Potrykus 1991). However, the number of isolated genes of agronomic importance is still quite limited because identifying and cloning the genes of interest are difficult. Thus, somatic cell hybridisation, which allows sexually incompatible plant species to be combined, including those belonging to different species, genera and tribes (Glimelius et al. 1991) is an alternative method of interest.

The transfer of DNA can be limited by irradiating the donor species protoplasts. This also increases the possibility of combining distantly related plant species via protoplast fusion (Dudits et al. 1987; Imamura et al. 1987; Gleba et al. 1988; Hinnisdales et al. 1988; Fahleson et al. 1994). Furthermore, irradiation has been found to induce intergenomic recombination events (Piastuch and Bates 1990; Feher et al. 1992; Parokonny et al. 1992), increasing the probability of obtaining the stable incorporation of alien DNA in the recipient genome.

The study presented here describes the production of intertribal somatic hybrid plants between *B. napus* and *L. fendleri*. These hybrids were made to exploit the *L. fendleri* germplasm for transfer of traits of agronomic importance to *B. napus*.

Materials and methods

Plant material

Brassica napus L. ssp *oleifera* cv 'Hanna' ($2n = 38$) and the wild species *Lesquerella fendleri* (Gray) Wats ($2n = 12$) were used as plant material for protoplast fusions. Seeds of *B. napus* were kindly provided by Svalöf Weibull AB, Sweden, while seeds of *L. fendleri* were obtained from the U.S. Department of Agriculture (USDA).

Protoplast isolation, fusion and culture

Isolated hypocotyl protoplasts of *B. napus* and mesophyll protoplasts of *L. fendleri* were used as material for the fusion experiments. The procedures used for protoplast isolation, fusion and selection of heteroplasmic fusion products have been described by Sundberg et al. (1987).

In the first set of experiments (the symmetric fusions), designated 1 to 4, which were performed to produce symmetric hybrids, protoplasts were fused without any pretreatment. In the second set of experiments (the asymmetric fusions), designated 5 and 6, the *L. fendleri* mesophyll protoplasts were irradiated before fusion. Irradiation was performed in W5 medium (Menczel et al. 1981) after isolating the protoplasts from the enzyme solution. For irradiation an X-ray Siemens Stabilipan 200 apparatus was used with doses of 180 Gy (experiment 5) and 200 Gy (experiment 6). Non-fused *L. fendleri* protoplasts were cultivated as a control in each fusion experiment to determine the effect of irradiation on the viability of the protoplasts. The culture of the hybrid cells and regenration of hybrid plants were performed according to Sundberg et al. (1987). Regenerated plants were transformed to hormone-free MS medium (Murashige and Skoog 1962) with 10% sucrose (MS-1), or to MS-1 medium with 0.1 mg/l NAA for root induction.

Analysis of *B. napus* (+) *L. fendleri* hybrid plants

Morphological analysis

Morphological characterisation of the plant material regenerated after fusion was performed at different developmental stages during growth and differentiation. The shape of the leaf primordia and the first fully developed leaves were used as the discriminating criteria for hybrid identification. On mature plants morphological analysis was made on the leaves and flowers as well as on the plant habit.

Nuclear and organelle DNA analysis

The presence of *B. napus* and *L. fendleri* nuclear DNA in putative hybrids was verified by performing Southern blot hybridisation using two species-specific repetitive DNA sequences. The *L. fendleri* repetitive sequence was isolated from total DNA partially digested with *Sau3*A according to Fahleson et al. (1994). The *B. napus* sequence was isolated by Liu et al. (1995) where randomly sheared and C-tailed total DNA was used for cloning. The organellar genomes were investigated with one heterologous chloroplast probe, a 4.6-kb *Sac*I fragment from lettuce (Jansen and Palmer 1987) and with two heterologous mitochondrial gene probes, the *atp*A and *cox*I gene. The mitochondrial gene probes were kindly provided by Dr. M. R. Hanson and Dr. C. S. Levings III.

The nuclear and organellar DNA analyses were performed on total DNA from putative hybrids and parental species that was isolated from leaves of *in vitro*-grown plants. The DNA isolation procedure was as described by Dellaporta et al. (1983) with modifications according to Forsberg et al. (1994). In total, 4–6 ug of DNA from each plant was digested with *Hin*dIII, separated by gel electrophoresis and transferred to nylon filters. Gel electrophoresis, Southern blots and hybridisations were performed as described by Landgren and Glimelius (1990), except that in the hybridisation solution 40% formamide was substituted for 6 *M* urea. The nuclear and organelle DNA probes were [^{32}P]-dCTP-oligolabelled according to the manufacturer's instructions using a DNA labelling kit (Pharmacia, Sweden).

Pollen viability and seed set

To calculate pollen viability we determined the percentage of pollen stained with acetoorcein by screening 1000 pollen grains from three flowers of each plant and calculating the average value. Seed set was determined as the number of seeds per pollinated flower. For each hybrid 50 flowers were cross-pollinated with *B. napus* and 50 flowers were self-pollinated.

Chromosome number analysis

Root tips were isolated from greenhouse-grown hybrid plants, and chromosome counting was performed according to Sundberg et al. (1987).

Results

Production of somatic hybrids

After polyethylene glycol-induced fusion the heteroplasmic fusion products were selected by flow cytometry and cell sorting, and the cells were cultured and regenerated into plants. The calli from the symmetric fusions were obtained from 13.9×10^4 selected fusion products, re-

sulting in a plating efficiency of 7.3%. In the asymmetric fusion experiments the plating efficiency decreased to 1.5% for the irradiation dose of 180 Gy and 2.5% for the dose of 200 Gy. In the control experiments, proliferation of the X-ray-irradiated *L. fendleri* mesophyll protoplasts in the dose of 180 Gy was significantly, but not totally, suppressed, while the dose of 200 Gy resulted in the complete inhibition of division.

Calli obtained from the selected fusion products were transferred to regeneration medium. Protocalli developed shoot primordia after 25–30 days and shoots were obtained after 35–40 days at frequencies differing significantly between the symmetric and the asymmetric fusion experiments (Table 1). Putative hybrid plants could be distinguished among the newly regenerated shoots and plantlets on the basis of the morphological cirteria. An intermediate or unique leaf shape and hairy leaves were considered to be hybrid chracteristics. Some hybrids were more densely covered with hairs than *B. napus*, but none of the putative hybrids was covered with *L. fendleri*-like trichomes. Furthermore, shoots that developed as a curled primary shoot were considered to be hybrid in character, since that feature was unique.

In total, 128 plants were classified as putative hybrids. A significant difference with respect to the frequency of hybrids regenerated from the symmetric and asymmetric fusions was found (Table 1). All plants obtained from the asymmetric fusions were categorised as hybrids while only 7% of the plants regenerated from the symmetric fusions were classified as hybrids. Fifty-three putative hybrids (S1–S53) were regenerated from the symmetric fusions, while from the asymmetric fusions 75 putative hybrids (As1–As75) were identified on the basis of the morphological criteria.

Nuclear and organellar genome analysis

The nuclear genome constitution was examined by analysing the presence of species-specific repetitive DNA sequences in plants that were classified as hybrids based on their morphological features (Table 2, Fig. 1). In total, 34 hybrids from the symmetric fusions and 46 from the asymmetric fusions were analysed. One plant from the symmetric fusions showed only a *L. fendleri*-specific nuclear DNA pattern, whereas 3 plants from the asymmetric fusions demonstrated a *B. napus*-specific pattern. All of the other plants had a hybrid-specific nuclear DNA with repetitive DNA sequences from both parental species present.

Organelle DNA analysis was carried out in all of the plants that were subjected to nuclear DNA examination. The chloroplast DNA (cpDNA) analysis revealed a non-random chloroplast segregation in favour of *B. napus* in hybrids from both the symmetric and the asymmetric fusions (Table 2, Fig. 1). The biased segregation of *B. napus* chloroplasts was even stronger in the hybrids regenerated from the asymmetric fusions. Of the plants originating from the asymmetric fusions almost all contained chloroplasts from *B. napus*; none had only *L. fendleri* chloroplasts, while 2 plants showed a biparental cpDNA pattern.

The segregation of mitochondria was also found to be biased, favouring *B. napus* mitochondria (Table 2, Fig. 1). This biased segregation of mitochondria was, similar to the chloroplast segregation, even stronger in the plants from the asymmetric fusions. Mitochondrial DNA (mtDNA) rearrangements were found in the hybrids. In total, 17 (50%) hybrids from symmetric fusions and 19 (41%) hybrids from asymmetric fusions exhibited rearranged mtDNA.

Morphology and fertility

Of the 53 putative hybrids regenerated from the symmetric fusions 5 (9%) could be established in the greenhouse (Table 3). From the asymmetric fusions, 24 (32%) plants, survived transfer to the greenhouse. Figure 2 shows the range of variation found in leaf and flower morphology among the greenhouse-grown plants. Even though a large number of plants displayed a morphology similar to that of *B. napus*, hybrid-specific characteristics were frequently found. For instance, plant S4 had *L. fendleri*-like elongated leaves (Fig. 2e) densely covered with hairs, which is uncommon both for *L. fendleri* and *B. napus*. The plant also had flowers with an abnormal morphology (Fig. 2k), with *L. fendleri*-like, globular pistils. The most common deviations from normal flower morphology found among the hybrids were rudi-

Table 1 Number of calli, shoots, regenerated plants and regeneration frequency in six *B. napus* (+) *L. fendleri* fusion experiments. In experiments 1–4 no pretreatments were used, while two different doses of X-rays (180 Gy and 200 Gy) were used to irradiate *L. fendleri* mesophyll protoplasts in experiments 5 and 6

Experiment number	Number of		Regeneration frequency (%)	Hybrid plants[a]	
	Calli	Shoots		Number	Frequency (%)
1–4	959	755	79	53	7
5	510	26	5	26	100
6	1268	49	4	49	100

[a] Hybrid plants were differentiated on the basis of morphological traits

Table 2 Nuclear, chloroplast and mitochondrial genome composition[a] of plants regenerated from the symmetric and the asymmetric *B. napus* (+) *L. fendleri* fusion experiments. The nuclear DNA was investigated with one species-specific repetitive DNA sequence from each parent. The organelle DNA analysis was performed with one chloroplast probe and two mitochondrial-specific probes

Fusion experiments	Number of plants	Nuclear DNA			Chloroplast DNA			Mitochondrial DNA			
		B	L	H	B	L	B+L	B	L	B+L	Rear
Symmetric	34	–	1	33	26	7	1	9	3	5	17
Asymmetric	46	3	–	43	44	–	2	27	–	–	19

[a] B, *Brassica*-specific DNA; L, *Lesquerella*-specific DNA; H, hybrid-specific rearranged DNA; B + L, sum of parental-specific fragments of cpDNA or mtDNA fragments, respectively; rear, rearranged mtDNA

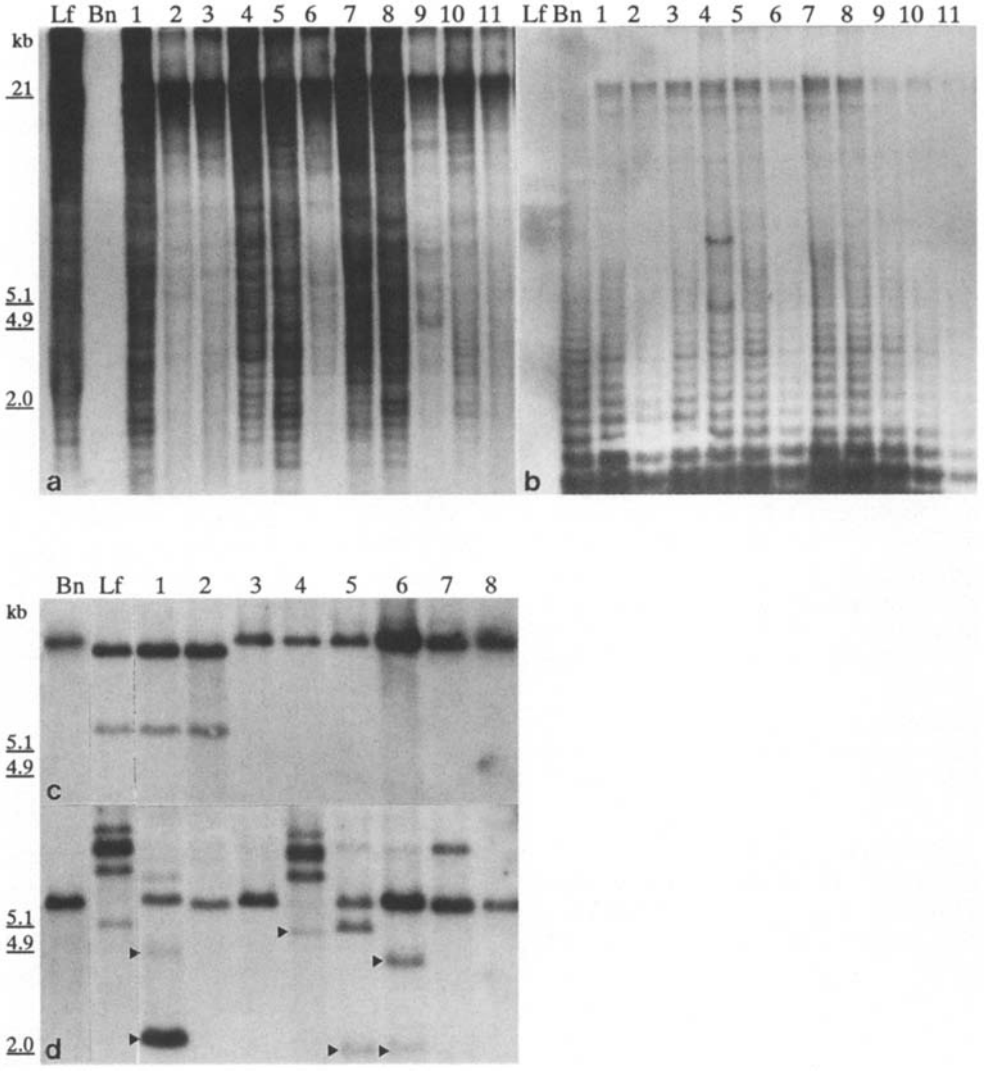

Fig. 1a–d Southern blot hybridisations of *Hin*dIII-digested total DNA from *B. napus* (+) *L. fendleri* somatic hybrids and the parental species. Hybridisation patterns with the nuclear species-specific repetitive DNA sequence from *L. fendleri* (**panel a**) and from *B. napus* (**panel b**): *lane Lf L. fendleri, Bn B. napus, 1–11 B. napus* (+) *L. fendleri* somatic hybrids. Hybridisation patterns with a heterologous chloroplast sequence, a 4.6-kb *Sac*I fragment (**panel c**), and with a *cox*I mitochondrial gene (**panel d**): *lane Lf. fendleri, Bn B. napus, 1–8 B. napus* (+) *L. fendleri* somatic hybrids. Hybrid-specific bands in mtDNA hybridisation patterns are marked by *arrows*. The molecular weight standard lambda DNA, cut with *Eco*RI/*Hin*dII, is indicated at the *left* (kb)

mentary or absent stamens, enlarged or distorted pistils, reduced or absent petals and small-sized flowers (Fig. 2i–l).

As shown in Table 3, none of the plants from the symmetric fusions were self-fertile, even though 3 plants had viable pollen. Only 2 plants (S1, S2) set seed after cross-pollination. Of the plants obtained from the asymmetric fusions 9 hybrids (As1, As2, As4–As6, As9–As12) could be selfed. Five additional plants (As3, As7, As8, As13, As14) produced viable pollen, but no seeds were obtained from 4 of these plants, either from self- or cross-pollination. However, plant As13, which had viable pollen and did not produce seeds after selfing, set seeds after cross-pollination. Ten plants (As15–As24) were male-sterile due to the production of non-viable pollen or to the lack of pollen production. For some plants, male sterility was due to the development of abnormal stamens. Plant As14 produced flowers with

Table 3 Chromosome numbers, morphology and fertility of *B. napus* (+) *L. fendleri* hybrid plants established in the greenhouse

Plant number[a]	Chromosome number	Morphological characteristics[b]				Pollen viability (%)	Seed set[d]	
		Anthers[c]	Pistils	Petals	Leaves		Selfed	x B.n
S1	50	N	B	small	B	5	–	1.8
S2	38	N	B	N	B	80	–	3.8
S3	38,44,50	N	B	N	B	16	–	0
S4	50	–	L	double	L	–	–	0
S5	50	rud	B	irreg	B	–	–	0
As1	38	irreg	distort	small	B	76	3.1	2.4
As2	38	N	B	N	B	52	1.4	2.4
As3	50,76	irreg	B	big	B	80	–	0
As4	38,46,48	–	distort	rud	B	–	–	0
As4*	38	N	B	N	B	47	0.8	0.9
As5	62	N	B	N	B	47	0.9	1.5
As6	38	N	B	small	B	60	0.2	1.3
As7	38	N	B	small	B/small	33	–	0
As8	38,42,50	small	long	small	B	40	–	0
As9	38	N	B	small	B	86	8.8	8.9
As10	38	N	B	N	B	62	2.0	1.4
As11	38	N	B	N	B	54	1.0	2.2
As12	38	N	B	small	B	60	5.4	7.2
As13	38	N	B	N	elong	81	–	0.7
As14 (graft)	nd	N	thin	small	B/small	58	–	0
As15	62	N	B	N	B	0	–	0.3
As16	76	–	long	small	B/small	–	–	0.4
As17	38	aborted flowers			elong	–	–	–
As18	62	–	long	rud	B	–	–	0
As19	50	aborted flowers			B/small	–	–	–
As20	76	–	long	rud	B	–	–	0.1
As21	38	–	distort	rud	elong	–	–	0
As22	48,76,84	small	thin	small	B/small	0	–	0.2
As23	50	aborted flowers			B	–	–	–
As24	62,76	aborted flowers			B/small	–	–	–
B. napus	38	N	B	B	B	100	8.3	nd
L. fendleri	12	N	L	L	L	80	6.4	nd

[a] S1-5, hybrids from symmetric fusions; As1-24, hybrids from asymmetric fusions; As4*, side branch from the original As4 hybrid. The chromosome number of As4* was determined in seedlings obtained after selfing

[b] N, Normal morphology; B, *B. napus*-specific morphology; L, *L. fendleri*-specific morphology; nd, not determined; rud, rudimentary anthers or petals; distort, thin, long, different types of abnormal pistil morphology; irreg, different length of anthers or size of petals; double, double flowers; elong, elongated but not *L. fendleri*-like leaves; 0, non-viable pollen; –, absence of anthers, pollen or seeds

[c] S5 had flowers with two anthers, whereas flowers of the other hybrids and of the parental species had six anthers

[d] Seed set was calculated as the number of seeds per pollinated flower

viable pollen only after grafting on *B. napus*. Hybrid As4 developed morphologically abnormal sterile flowers. However, after reaching maturity, one side-branch of this plant produced fertile *B. napus*-like flowers.

Chromosome number analysis

Chromosome number was examined in all plants but 1 that were established in the greenhouse (Table 3). Three of the hybrids (S1, S4, S5) from the symmetric fusions contained 50 chromosomes, which corresponds to the sum of the parental genomes. Eleven hybrids (As1, As2, As6, As7, As9–As13, As17, As21) from the asymmetric fusions contained 38 chromosomes; 2 plants (As19, As23) had 50 chromosomes and 5 plants (As5, As15, As16, As18, As20) exhibited chromosome numbers higher than the sum of the parental genomes. In total, 6 hybrids (S3, As3, As4, As8, As22, As24) were found to have cells with varying chromosome numbers in different cells of the root tips.

Discussion

In the present investigation we have shown that *B. napus* and *L. fendleri* are somatically compatible and can be combined into hybrid plants even though these species belong to different tribes in the Brassicaceae family. Hybrid plants which produced viable seeds were obtained.

Many of the regenerated plants obtained in the symmetric fusion experiments were identical to *L. fendleri*. The protoplast-derived colonies of *L. fendleri* appeared to differentiate into plantlets via an embryogenic pathway. The development was rapid; the *L. fendleri* protoplasts regenerated and differentiated into shoots after 30–35 days at a frequency of about 90% (data not

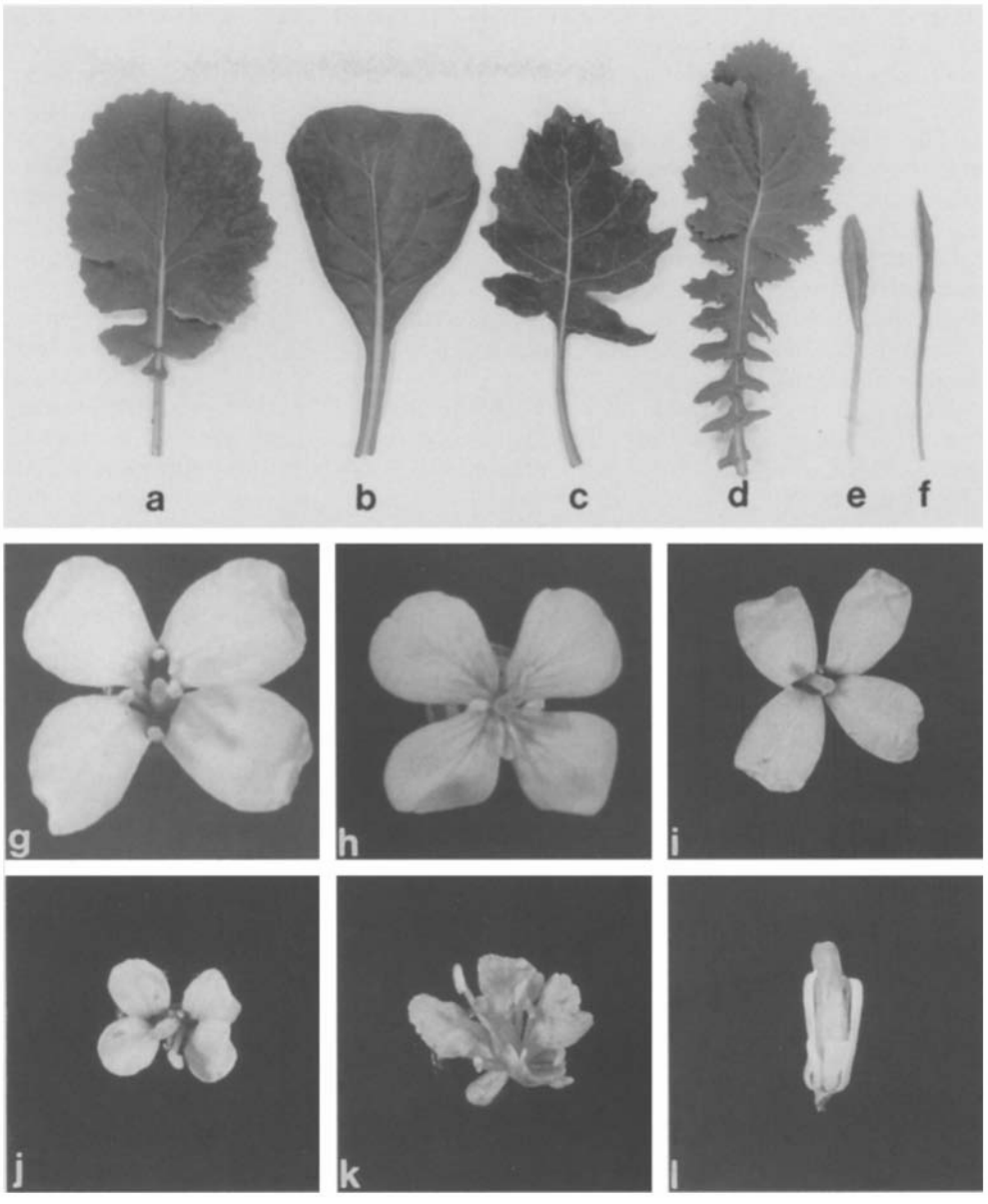

Fig. 2 Plant morphology of *B. napus* (+) *L. fendleri* somatic hybrids and parental species. Leaves: *a B. napus*, *b* As3, *c* As4, *d* As13, *e* S4, *f L. fendleri*; and flowers: *g B. napus*, *h L. fendleri*, *i* As8, *j* As1, *k* S4, *l* As20

shown). In contrast, protoplast-derived shoots of *B. napus* were usually obtained after 45–50 days in culture via an organogenic pathway at a frequency of about 20–30% (Glimelius 1984). Thus, the high frequency of *L. fendleri*-like plants obtained in the symmetric fusion experiments could be due to the high proliferation rate and regeneration capacity of *L. fendleri* protoplasts that escaped the selection procedure. According to protoplast fusion experiments performed previously in our laboratory, selection efficiency using flow cytometry and cell sorting is about 70–80% (Glimelius et al. 1986; Fahleson et al. 1994; Liu et al. 1995).

Alternatively, the regeneration of *L. fendleri* plants in the symmetric fusion experiments could be caused by the elimination of *B. napus* genetic material from heteroplasmic fusion products. The genetic potential for protoplast division, callus proliferation and regeneration of the individual parental genotypes is thought to affect the production of somatic hybrid plants (Dudits et al. 1987; Gleba et al. 1987, 1988; Lelivelt et al. 1993). The first division of both *L. fendleri* mesophyll cells and *B. napus* hypocotyl protoplasts occurred after 2–3 days in culture. However, even though protoplasts from both species started to divide synchronously, differences in the proliferation activity were recognised, that were most probably due to differences in cell-cycle rates. It has been proposed that differences in cell-cycle phase, cell-cycle duration and ploidy level of parental protoplasts are responsible for chromosome elimination in remote hybrids (Bennett et al. 1976; Kao 1977; Gleba and Sytnik 1984; Sundberg and Glimelius 1991). However, by utilising morphological characters, we could easily select hybrid plants from the parental *L. fendleri* plants regenerated from the symmetric fusions. This selection was

clearly confirmed by the nuclear DNA analysis, since 33 out of 34 plants selected on morphological criteria to be hybrids contained species-specific DNA sequences from both parents.

The irradiation had no influence on the proliferation activity of the heterokaryon-derived cells. However, it drastically reduced growth and the morphogenic potential of the irradiated protoplasts and, consequently, the total number of regenerants. Furthermore, irradiation resulted in a preferential selection of hybrids, since most of the plants which regenerated from the asymmetric fusions had DNA from both parents based on the nuclear DNA analysis.

Major efforts were made to establish the somatic hybrids in the greenhouse. By partially eliminating *L. fendleri* DNA using irradiation, we increased our possibilities of obtaining mature *B. napus* (+) *L. fendleri* plants. Furthermore, irradiation of the *L. fendleri* protoplasts improved the fertility of the *B. napus* (+) *L. fendleri* hybrids. All of the plants from the symmetric fusions were self-sterile, while 9 (38%) of the asymmetric hybrids could be selfed. In contrast, Fahleson et al. (1994) did not find any positive effects of irradiation on the fertility of intertribal hybrids between *B. napus* (+) *Thlaspi perfoliatum*, although it did improve the efficiency of hybrid production. Hybrids were obtained from both the symmetric and the asymmetric fusions that did not set seed either after selfing or cross-pollination even though they had viable pollen, which indicates that these hybrids were female-sterile. Moreover, some plants with viable pollen produced seeds after cross-pollination but not after selfing, which might be due to self-incompatibility.

The fact that several of the hybrids from both the symmetric and asymmetric fusions had normal flower morphology demonstrates that the *B. napus* and *L. fendleri* genomes are compatible. It is therefore unlikely that the morphological abnormalities found only reflect a nuclear intergenomic incompatibility. It is also unlikely that a nuclear cytoplasmic incompatibility was the major factor causing abnormal flower morphology, since no correlation was found between mitochondrial genotype and flower phenotype (data not shown). However, abnormal flowers were found frequently in plants from the asymmetric fusion experiments. These abnormalities could be a result of chromosomal instability in biparental cells (Clark et al. 1986; Pehu et al. 1989). Moreover, Bauer-Weston et al. (1993) suggested that flower abnormalities could be caused by irradiation-induced mutations in some flower-specific genes. Alternatively, irradiation of one of the fusion partners might lead to changes in the genome organisation of the other partner, as reported by Kovtun et al. (1993). Thus, irradiation of *L. fendleri* protoplasts prior to fusion could have induced the deviations from normal flower morphology observed in the hybrids.

Organellar DNA analysis of the hybrid plants in this study showed a biased segregation, favouring *B. napus* organelles. This corresponds with results obtained earlier in our laboratory from cpDNA and mtDNA studies of hybrids of several other species combinations within Brassicaceae (Landgren and Glimelius 1990, 1994; Sundberg and Glimelius 1991; Sundberg et al. 1991; Fahleson et al. 1994; Forsberg et al. 1994). Nuclear DNA content and cell size both influence plastid number and cpDNA content (Butterfass 1988), which might lead to an unequal input of chloroplasts in the hybrid cell. The non-random segregation of *B. napus* chloroplasts found in this study could thus reflect an unequal input of chloroplsts in the hybrid cell due to the fact that allotetraploid *B. napus* was combined with *L. fendleri*, which is diploid. A correlation between chloroplast segregation pattern and the degree of genetic distance between combined species has also been demonstrated (Perl et al. 1991; Sundberg and Glimelius 1991). Since *B. napus* and *L. fendleri* belong to different tribes within the Brassicaceae family the biased chloroplast segregation could reflect a restricted nuclear-plastid compatibility in this intertribal species combination or it could reflect differences in plastid multiplication rates, as proposed by Bonnett and Glimelius (1983) and by Levi et al. (1988).

The biased mitochondrial segregation found in the *B. napus* (+) *L. fendleri* hybrids is in accordance with results obtained in our laboratory from mtDNA analyses of a large number of different *Brassica* somatic hybrids (Landgren and Glimelius 1994; Landgren et al. 1994). These results indicate that the *B. napus* mitochondria are the most competitive, perhaps due to such inherent characteristics as a higher multiplication rate. However, the possibility cannot be ruled out that the biased organelle segregation is an effect of the culture and regeneration media that were developed for *B. napus* (Glimelius 1984).

This investigation indicated that irradiation affected organelle segregation, since the bias in favour of *B. napus* organelles was even stronger in hybrids from the asymmetric fusions, none of which contained *L. fendleri*-specific chloroplasts and/or mitochondria. Bonnema et al. (1992) reported similar results but were unable to deduce whether the alterations in organelle inheritance were a direct result of irradiation or an effect of the nuclear background, the latter being affected by the irradiation treatment. However, several studies have indicated that the nuclear background of the hybrids has an effect on organellar segregation (Sundberg and Glimelius 1991; Wolters et al. 1991, 1993). The stronger bias favouring *B. napus* organelles in hybrids from the asymmetric fusions in the present study might thus reflect the greater reduction in the *L. fendleri* nuclear genome due to the X-ray irradiation.

Chromosome studies revealed that 3 out of 5 plants from the symmetric fusions contained the chromosome number corresponding to the sum of the parental species. In contrast, hybrid S2 contained 38 chromosomes, which corresponds to the chromosome number of *B. napus*. This genome constitution could be due to elimination of the *L. fendleri* genetic material. However, nu-

clear DNA analysis showed that this plant contained *L. fendleri*-specific DNA sequences, which could mean that intergenomic rearrangements occurred. Such rearrangements have been shown in somatic hybrids produced without irradiation (White and Rees 1985; Parokonny et al. 1992). Alternatively, a spontaneous biparental (but incomplete) chromosome elimination could have taken place in hybrid S2. This could also explain the presence of *L. fendleri* as well as *B. napus* DNA in the hybrid genome. Since the number of chromosomes contributed by each parent in the hybrid genome could not be determined by means of ordinary squash technique because of the similarity in the sizes of the chromosomes of the two species, it was not possible to verify the species-specific chromosome constitution.

Several hybrids from the symmetric fusions possessed 38 chromosomes, which most probably was a result of irradiation-induced chromosome elimination in the heteroplasmic fusion products. Hybrids with chromosome numbers greater than the sum of the parental species could derive from multiple fusion or from endoreduplication of one of the parental genomes after fusion, as has been discussed by Sundberg et al. (1991). In this study the presence of plants having cells with different chromosome numbers in the root tips indicates that chromosome instability and/or hybrid genome reorganisation also occurs in mature plants. From studies of potato somatic hybrids it was suggested that a continuos and prolonged process of chromosome elimination results in a stepwise improvement in regeneration and differentiation into morphologically normal plants (Feher et al. 1992). In the present study, a somatic segregant, plant As4*, was found. In the original hybrid (As4), cells with 38, 46 and 48 chromosomes were present. The primary hybrid had morphologically and functionally abnormal flowers but subsequently developed fertile *B. napus*-like flowers on a side branch from which seeds were obtained; the germinated seeds were found to have 38 chromosomes. This finding may support the idea of a selection process promoting chromosome elimination during the differentiation and vegetative growth of somatic hybrids.

In summary, intertribal somatic hybrids of *B. napus* (+) *L. fendleri* were obtained from both the symmetric and the asymmetric fusion experiments. However, by using X-ray irradiation in the fusion procedure we obtained an increased compatibility between the two remote genomes. Larger numbers of hybrid plants were recovered from the asymmetric fusions than from the symmetric fusion experiments. Furthermore, X-ray irradiation improved the fertility of hybrid plants, and hybrid progenies were obtained from the asymmetric fusions. Consequently, it will be possible to trace the inheritance of parental-specific traits of interest and to study the genome constitutions of these hybrids.

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