Theor Appl Genet (2003) 106:1147–1155
DOI 10.1007/s00122-002-1176-x

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Development of rapeseed with high erucic acid content by asymmetric somatic hybridization between *Brassica napus* and *Crambe abyssinica*

Received: 24 May 2002 / Accepted: 15 October 2002 / Published online: 10 April 2003

Abstract PEG-induced asymmetric somatic hybridization between *Brassica napus* and *Crambe abyssinica* was carried out. *C. abyssinica* is an annual cruciferous oil crop with a high content of erucic acid in the seed oil valuable for technical purposes. UV-irradiated mesophyll protoplasts of *C. abyssinica* cv 'Carmen' and cv 'Galactica' were fused with hypocotyl protoplasts of different genotypes of *B. napus* cv 'Maplus' and breeding line '11502'. Shoot regeneration frequency varied between 6.1% and 20.8% among the different doses of UV-irradiation, ranging from 0.05 J/cm^2 to 0.30 J/cm^2. In total, 124 shoots were regenerated, of which 20 asymmetric somatic hybrids were obtained and verified by nuclear DNA content and AFLP analysis. AFLP data showed that some of the characteristic bands from *C. abyssinica* were present in the hybrids. Cytological analysis of these hybrids showed that 9 out of 20 asymmetric hybrids had 38 chromosomes, the others contained 40–78 chromosomes, having additional chromosomes between 2 and 40 beyond the 38 expected for *B. napus*. The investigation into the fertility of asymmetric somatic hybrids indicated that the fertility increased with increasing UV-doses ranging from 0.05 J/cm^2 to 0.15 J/cm^2. All of the hybrids were cultured to full maturity, and could be fertilized and set seeds after self-pollination or backcrosses with *B. napus*. An analysis of fatty acid composition in the seeds was conducted and found to contain significantly greater amounts of erucic acid than *B. napus*. This study indicates that UV-irradiation could be used as a tool to produce asymmetric somatic hybrids and to promote the fertility of the hybrids.

Communicated by K. Glimelius

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Keywords *Brassica napus* · *Crambe abyssinica* · Somatic hybridization · UV-irradiation · High erucic acid rapeseed

Introduction

Oilseed rape (*Brassica napus*) has become the world's third most important annual crop for vegetable oil production due to substantial progress in breeding and cultivation practices. In order to make rapeseed oil more competitive in various segments of the food and industrial oil markets, modification of the fatty acid composition has been an important objective of plant breeding in recent years (Friedt and Lühs 1998). Erucic acid ($C_{22:1}$) is a very important raw material of industry and can be used as an additive to lubricants and solvents, a softener for textiles and an amide derivative in polymer synthesis (Mastebroek and Lange 1997). It naturally occurs in high erucic acid rapeseed (*B. napus*) varieties and other *Brassicaceae*. The canola-type *B. napus* cultivars grown for human consumption, however, have a low content of erucic acid. *Crambe abyssinica*, belonging to the tribe *Brassiceae*, is an annual herb with a high content of erucic acid (55%–60%) in the seed oil. Moreover, *C. abyssinica* possesses some valuable characteristics, such as a short growing period, wide adaptation (Wang et al. 2000) and insect resistance (Anderson et al. 1992; Kmec et al. 1998), making it a potential gene donor for the modification of *B. napus*.

Somatic hybridization has developed into a promising technique for the introgression of alien genes into a domesticated crop, and facilitates the transfer of nuclear-encoded as well as organelle-encoded genes between plant species. However, some problems usually exist with the heredity of symmetric somatic hybrids between distant species, such as incompatibility of genome recombination and infertility. In order to transfer only a limited part of a genome of one species (donor) to the genome of another species (recipient), the technique of asymmetric somatic hybridization has been developed

(Dudits et al. 1980). Many studies show that asymmetric somatic hybrids often have a higher regeneration and rooting capacity as well as increased fertility compared to symmetric hybrids (Bates et al. 1987; Fahleson et al. 1994; Skarzhinskaya et al. 1996; Forsberg et al. 1998b).

In the *Brassicaceae*, most species are amenable to protoplast-culture and plant-regeneration techniques (Brewer et al. 1999). *B. napus* has been fused with plants of many other genera, including *Eruca sativa* (Fahleson et al. 1988), *Diplotaxis harra* (Klimaszewska and Keller 1988), *Sinapis alba* (Primard et al. 1988; Hansen and Earle 1997), *Raphanus sativus* (Sundberg and Glimelius 1991), *Arabidopsis thaliana* (Forsberg et al. 1994), *Thlaspi perfoliatum* (Fahleson et al. 1994), *Lesquerella fendleri* (Skarzhinskaya et al. 1996), *Moricandia arvensis* (O'Neil et al. 1996) and *Thlaspi caerulescens* (Brewer et al. 1999). Protoplast fusion was used to transfer organellar traits (Jourdan et al. 1989; Arumugam et al. 2000), a trait for metal hyperaccumulation (Brewer et al. 1999) and disease resistance (Gerdemann-Knörck et al. 1995; Ren et al. 2000). Some efforts have been made to modify the fatty acid composition of rapeseed through protoplast fusion. Heath and Earle (1995, 1997) synthesized novel *B. napus* with a high content of erucic acid (57.4%) and a low content of linolenic acid (3.5%) through protoplast fusion. Fahleson et al. (1994) produced intertribal somatic hybrids between *B. napus* and *T. perfoliatum* with a higher content of the nervonic acid (4.9%) than that of *B. napus*.

Pre-fertilization incompatibility in sexual hybridization was observed in crosses of *B. napus* and *C. abyssinica* (Wang and Luo 1998). To our knowledge, no somatic hybridization between *B. napus* and *C. abyssinica* has been reported so far. In the present study, we have investigated UV-irradiation as a tool in the production of asymmetric somatic hybrids between *B. napus* and *C. abyssinica*. Our approach was to fuse UV-irradiated mesophyll protoplasts of *C. abyssinica* with hypocotyl protoplasts of different genotypes of *B. napus*. The objectives of this study are: (1) to create novel somatic hybrids through protoplast fusion of *B. napus* and *C. abyssinica*, (2) to recover somatic hybrids with a higher erucic acid content than *B. napus* and confirm the hybridity of the regenerants by morphology, molecular marker and cytology, and (3) to promote the fertility of asymmetric somatic hybrids by UV-irradiation of the donor parent.

Materials and methods

Plant materials

Seed of *B. napus* (2n = 38) cv 'Maplus' was kindly provided by the Norddeutsche Pflanzenzucht, Hans Georg Lembke KG (Germany), and breeding line '11502' by the Plant Breeding Company KWS Saatzucht AG (Germany). Seed of *C. abyssinica* (2n = 90) cv 'Carmen' was obtained from Cebeco Seeds GmbH (Germany), and cv 'Galactica' from the Centre for Plant Breeding and Production Research, CPRO-DLO (The Netherlands).

Protoplast isolation, UV-irradiation, fusion and cell culture

Seeds of both species were surface-sterilized in 70% alcohol for 2 min and 3% (v/v) commercial bleach for 25 min, and then rinsed in sterile water. The seeds of *B. napus* were germinated on half-strength MS medium (Murashige and Skoog 1962) at 25 °C in the darkness to produce hypocotyls. In vitro cultivated plants of *C. abyssinica* were grown on MS medium and the leaves were harvested to yield mesophyll protoplasts for the protoplast-fusion experiments.

Five to seven-day old hypocotyls of *B. napus* and 3–4 week-old leaves of *C. abyssinica* were harvested, cut into small pieces, and preincubated for 20 min in a solution containing sorbitol 54.6 g/l and $CaCl_2 \times 2H_2O$ 7.4 g/l (pH 5.6), and then incubated in a mixture of enzymes (0.25% cellulase 'Onozuka R-10', 0.05% macerozyme R-10) with 0.6 M mannitol overnight at 25 °C in the darkness. Protoplasts were purified by filtering (using a 50-μm sieve), centrifugating (1,000 rpm × 5 min) and washing (0.154 M NaCl, 0.125 M $CaCl_2 \times 2H_2O$, 0.005 M KCl, 0.005 M glucose, pH 5.8) and then diluted to a concentration of 1×10^6/ml with medium CR [MS medium + 150 mg/l of caseinhydrolysat, 875 mg/l of $CaCl_2 \times 2H_2O$, 45.5 g/l of sorbitol, 45.5 g/l of mannitol, 2.5 g/l of D-glucose, 125 mg/l of D-ribose and 0.5 mg/l of 2,4-D, 0.2 mg/l of naphthaleneacetic acid (NAA), 0.2 mg/l of 6-benzylaminopurine (6-BA), pH 5.8]. The protoplasts of *C. abyssinica* (donor) were exposed to different doses of UV with a wavelength of 254 nm prior to fusion in 'Biometra' UV equipment. The doses used were 0.05, 0.075, 0.10, 0.15, 0.20, 0.30 J/cm^2. As a control, one fusion experiment in which the *C. abyssinica* protoplasts were not exposed to UV-irradiation was also included in this study. The *Brassica* protoplasts and UV-irradiated donor protoplasts were then mixed in a ratio of 1:1.2. Fusion was induced with 40% polyethylene glycol (PEG) according to the method of Ryschka et al. (1996). PEG-treated protoplasts were cultured in CR medium at 25 °C in darkness. The same medium was added every 5 days until microcalli reached a size of 32–64 cells.

Plant regeneration

After 2–3 weeks, microcalli formed in CR medium. Then they were transferred onto a solid medium [MS medium + 10 g/l of sucrose, 20 g/l of mannitol, 250 mg/l of caseinhydrolysat, 6 g/l of agarose, 0.5 mg/l of NAA, 0.5 mg/l of 6-BA, 1 mg/l of TDZ (thidiazuron)] and cultured at 24 °C under cool-white fluorescent light with a 16-h photoperiod. Four weeks later, they were transferred to a differentiation medium (MS medium + 20 g/l of sucrose, 250 mg/l of caseinhydrolysat, 1.0 mg/l of 6-BA, 0.04 mg/l of gibberellic acid, 10 g/l of agar). Shoots emerged after 3–4 weeks and then they were transferred to a MS medium without hormones for plantlet development.

Nuclear DNA content

A total of 0.5 cm^2 of fresh leaves of the parental genotypes and putative somatic hybrids were cut into pieces in 0.25 ml of nuclei isolation solution A (Partec GmbH) and then 1 ml of DAPI staining solution B was added. The suspension was immediately filtrated through a 30-μm filter and incubated for 20 min at 4 °C, and then analyzed by flow cytometry with a 'Partec Cell Analyzer CA-II' (Partec GmbH, Germany).

AFLP analysis

Total genomic DNA was extracted from young leaves of the fusion parents and regenerated plants according to the instruction of the DNeasy Plant Mini Kit (Qiagen, Germany). AFLP analysis was performed according to Vos et al. (1995) using the AFLP Analysis System I (Life Technology, Germany). Fragments were separated

for 3–4 h at 70 W on standard sequencing gels and visualized by silver staining.

Pollen viability and hybrid fertility

Pollen viability was determined by squashing anthers 1 day after anthesis in a drop of 1% acetocarmine and counting several fields of 500 pollen grains under a microscope at 200×. Pollen grains that appeared plump, round and stained red were counted as viable, while pollen grains that appeared shrivelled and unstained were not. For each hybrid plant, flowers were self-pollinated or backcrossed with *B. napus*. Fertility (seed set) was measured by the number of seeds obtained per pollinated flower.

Chromosome counts

To determine the number of chromosomes in somatic cells, root tips from the hybrids were pretreated with 2 mM of 8-hydroxyquinoline for 4 h, and fixed in Carnoy's solution (ethanol: glacial acetic acid = 3:1). The tips were then hydrolyzed in 1 N HCl at 60 °C for 5 min and stained with modified carbol fuchsin. For meiotic observation, young flower buds from the hybrids were also fixed in Carnoy's solution and then squashed and stained with aceto-carmine.

Fatty acid analysis

Seeds from the parents and the hybrid plants were subjected to analyses of the fatty acid composition. For each of the 20 hybrid plants and their parents, ten seeds harvested in the same greenhouse were chosen for fatty acid analysis. The fatty acids were determined as fatty acid methyl ethers (FAME) by capillar gas chromatography according to the method of Thies (1971). Each seed was homogenized separately in t-butylmethyl ether to dissolve the storage lipids. Derivative preparation into FAMEs was done with trimethylsulfoniumhydroxide (TMSH) solution. The chemical analysis was performed with a gas chromatograph HP 5890 with an autosampler HP 7673 (both of HewlettPackard) according to a temperature program. An FFAP column with a 25-m length, 0.25 mm of ID, and 0.3 μm of FD was used. The calibration was performed with FAME standard mixtures of Supelco. Fatty acids with chain lengths between C_{14} and C_{24} were determined. The content of the several fatty acids is given as a percentage of the total sum of detected fatty acids. Statistical tests of erucic acid content are based on the measurement of the ten single seeds (i.e. ten replications) per hybrid plant and the parents, respectively. They are performed with SAS/STAT Software release 6.12 (SAS Institute Inc.)

Results

Protoplast culture and plant regeneration

Mesophyll protoplasts of *C. abyssinica* (Fig. 1a) were irradiated using different doses of UV light prior to fusion with hypocotyl protoplasts of *B. napus* (Fig. 1b). UV-irradiated mesophyll protoplasts of *C. abyssinica* were fused with hypocotyl protoplasts of *B. napus* by the PEG-induced method (Fig. 1c). Unfused *C. abyssinica* protoplasts were not able to divide and proliferate in the CR medium because a lethal dose of UV treatment prevented their cell division. The first division of the cultured protoplasts was observed on the 3rd–4th day after fusion (Fig. 1d). Microcalli visible with the naked eyes formed after 2–3 weeks' culture (Fig. 1e,f). When grown to about 2 mm in size, they were transferred to a solid medium. Shoots emerged 3–4 weeks later (Fig. 1g) and were transferred to MS medium. Shoot regeneration frequency varied between 6.1% and 20.8% among the different doses of UV-irradiation ranging from 0.05 J/cm^2 to 0.30 J/cm^2, and a total of 124 shoots was regenerated (Table 1). The frequency of asymmetric hybrid plants varied between 20.0% and 33.3% in the experiments where different UV-irradiation doses (0.05 J/cm^2–0.15 J/cm^2)

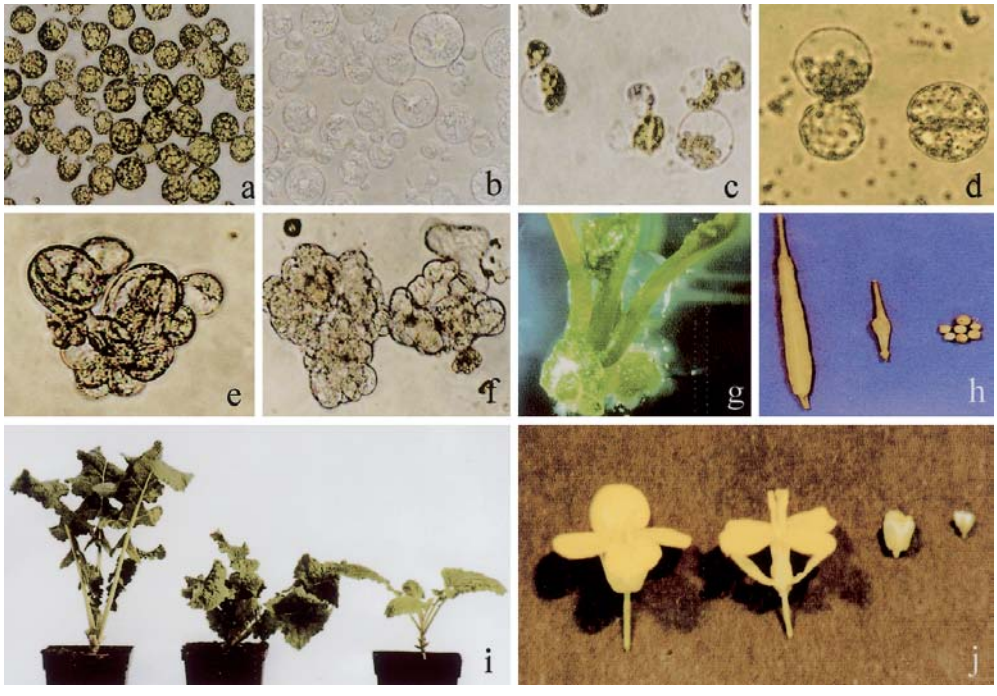

Fig. 1 (**a**) Mesophyll protoplasts of *C. abyssinica*. (**b**) Hypocotyl protoplasts of *B. napus*. (**c**) Fused protoplasts. (**d**), (**e**) Cell division of the fused protoplast. (**f**) Microcalli. (**g**) Calli with shoot. (**h**)–(**j**) Comparison of silique, young plants and flowers of a hybrid, and its fusion partners. Left, *B. napus*; middle, hybrid; right, *C. abyssinica*

Table 1 Effect of different doses of UV-irradiation on the frequency of regenerated shoots and hybrid plants

UV-irradiation dose (J/cm^2)	No. of induced calli	Regenerated shoots		Hybrid plants	
		Number	Frequency[a] (%)	Number	Frequency[b] (%)
0.00	1,021	58	5.7	0	0
0.05	48	10	20.8	2	20.0
0.075	272	24	8.8	8	33.3
0.10	479	29	6.1	9	31.0
0.15	33	3	9.1	1	33.3
0.20	45	0	0	0	0
0.30	6	0	0	0	0
Total	1,904	124		20	0

[a] No. of regenerated shoots/no. of induced calli
[b] No. of hybrid plants/no. of regenerated plants

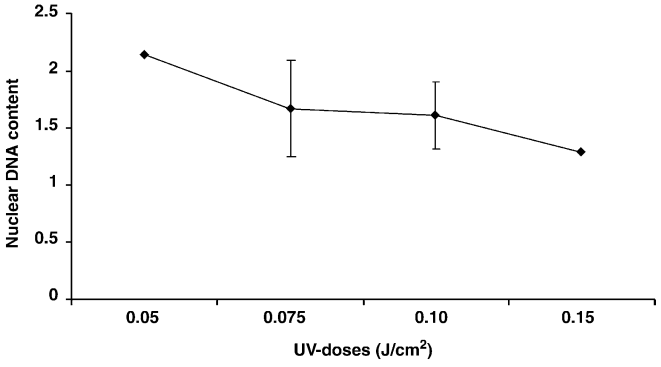

Fig. 2 Effects of UV doses on the nuclear DNA content of the asymmetric somatic hybrids (bars indicate standard error)

were used as pretreatment. No shoots were regenerated from fusions with *C. abyssinica* protoplasts irradiated with more than a 0.20 J/cm^2 UV dose.

Selection of putative hybrids by flow cytometry

In total, 124 shoots were regenerated and all of them were tested by nuclear DNA content analysis. The nuclear DNA content of plants derived from asymmetric somatic fusion varied considerably, thus confirming the results of other studies (Babiychuk et al. 1992; Fahleson et al. 1994). The plants were assigned to one of three different classes as follows: (1) a nuclear DNA content corresponding to the DNA content of *B. napus* (recipient), (2) a nuclear DNA content greater than that of *B. napus* but less than the sum of *B. napus* and *C. abyssinica*, and (3) a nuclear DNA content greater than the sum of the parental content. The 42 plants of the second class were accounted as putative hybrids, and further confirmed by AFLP analysis. Figure 2 shows that the nuclear DNA content of asymmetric somatic hybrids was decreased with increasing doses of UV-irradiation ranging from 0.05 J/cm^2 to 0.15 J/cm^2.

Confirmation of hybrids by AFLP analysis

After the pre-selection by flow cytometry, 42 putative hybrids were obtained. AFLP analysis was carried out to confirm their hybridity. AFLP assays of parental genomic DNA produced a characteristic banding pattern for each species. The greatest number of discriminating bands was produced by *Eco*RI-AAC/*Mse*I-CGA (Fig. 3). Fourteen bands were present only in *C. abyssinica* (lane 22, indicated by arrows), while *B. napus* exhibited 13 unique bands (lane 1) as shown in Fig. 3. Of the 42 putative hybrid plants, 22 plants displayed only the *B. napus* AFLP DNA pattern, while 20 showed the *B. napus* pattern containing at least one unique band characteristic of *C. abyssinica* (lanes 2–21), thus proving that part of the genetic material from *C. abyssinica* (donor) was transferred into *B. napus* (recipient). Some of the hybrids contained only a small proportion of donor DNA; for example, hybrid nos. 4, 6, 7, 8, 9 and 11 (lanes 5, 7, 8, 9, 10 and 12) presented only one band from *C. abyssinica*. Most of hybrids had additional bands which were not present in *B. napus* and *C. abyssinica* (Fig. 3). Significant variation was also found between different hybrids. When the UV dose was 0.05 J/cm^2, 8–10 bands were present (lanes 20, 21), while UV doses ranging from 0.075 J/cm^2 to 0.15 J/cm^2 resulted in bands ranging from 1 to 7. The results show that the lower UV-irradiation dose was applied to the donor protoplast, and more bands characteristic of *C. abyssinica* are present in the hybrids. The result is verified by a negative correlation coefficient of $r = -0.48$ ($P < 0.05$). It indicates that the degree of asymmetry could be increased with the application of high doses of UV-irradiation to the donor protoplast, and a highly asymmetric somatic hybrid could be produced.

Morphology, pollen viability and seed set of hybrids

In many respects the F_1 hybrids resembled *B. napus*. At the seedling stage, the basal leaves of the hybrids were ovate like *C. abyssinica* (Fig. 1i). The flowers were smaller than *B. napus* but larger than *C. abyssinica* (Fig. 1j). The petals were creamy yellow like *B. napus*, whose petals are yellow, and unlike those of *C. abyssini-*

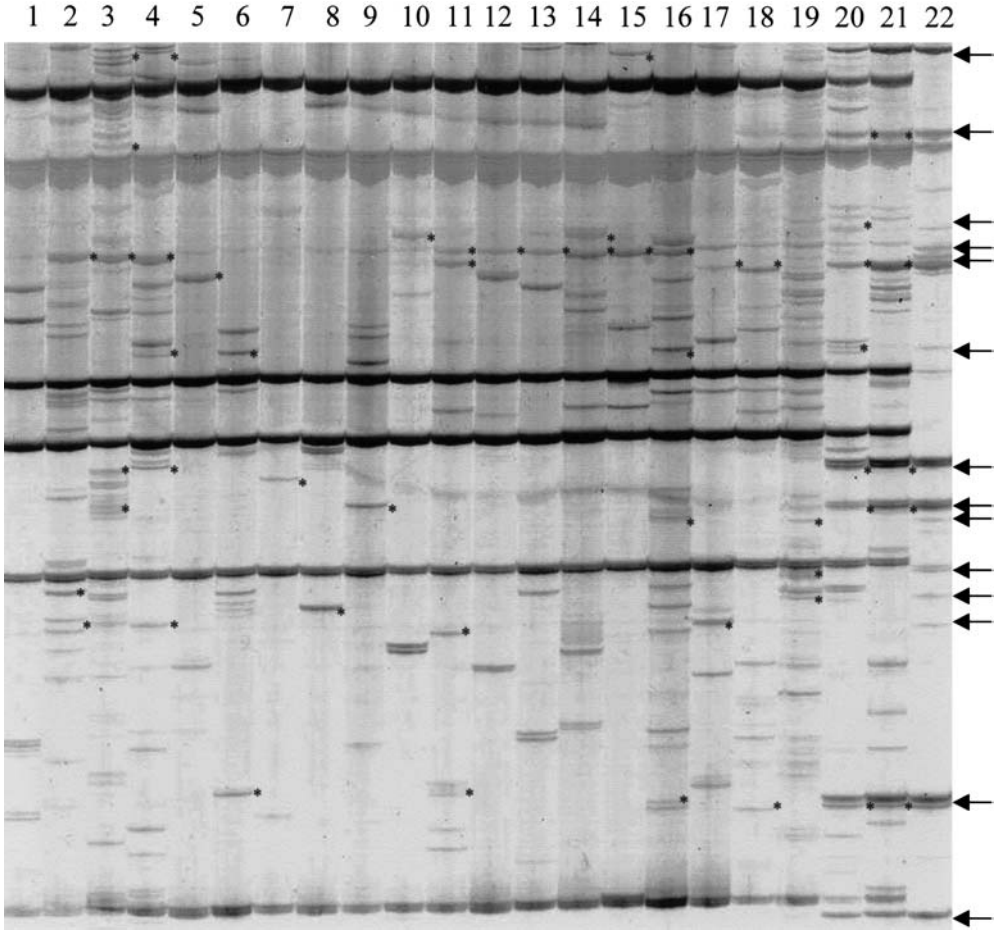

Fig. 3 AFLP banding patterns using the primer pair E-AAC/M-CGA of the *B. napus* plant (*lane1*), somatic hybrids (*lanes 2–21*) and the *C. abyssinica* plant (*lane 22*). Fourteen bands characteristic of C. *abyssinica* are indicated with *arrows*, specific bands present in the hybrids from *C. abyssinica* are indicated with an *asterisk* (*)

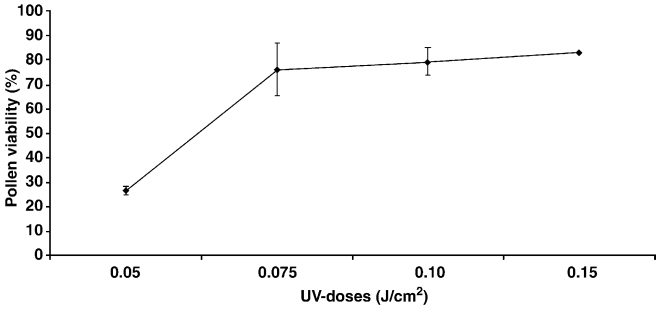

Fig. 4 Effects of UV doses on pollen viability of the asymmetric somatic hybrids (bars indicate standard error)

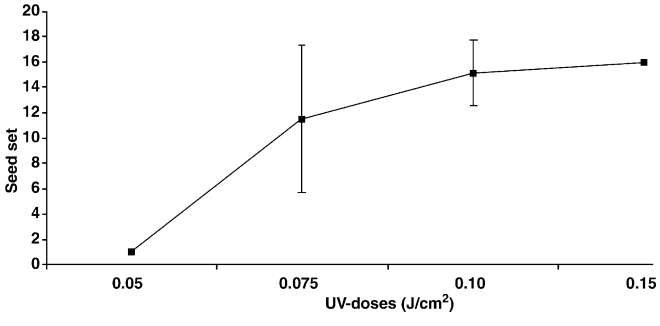

Fig. 5 Effects of UV doses on seed set of the asymmetric somatic hybrids (bars indicate standard error)

ca, whose petals are white. The F_1 plants had a smaller silique compared to *B. napus* (Fig. 1h). Seed set was achieved under self-pollination or in backcrosses with *B. napus*. Most of the seeds harvested from the hybrids were round in shape and larger in size than that of *B. napus*. The pollen viability of the 20 hybrids varied between 25% and 90% (Fig. 4). If UV-irradiation was 0.05 J/cm^2, low pollen viability (about 25%) and low seed set (one seed per pollinated flower) were obtained. If UV-irradiation was from 0.075 J/cm^2 to 0.15 J/cm^2, the hybrids had higher pollen viability varying from 62% to 90%, and more seeds were set in the hybrids. This indicates that increasing UV-irradiation doses (0.075 J/cm^2 to 0.15 J/cm^2) result in a higher pollen viability as well as a higher seed set (Figs. 4, 5).

Analysis of chromosome number

Chromosome numbers were determined utilizing a combination of mitotic and meiotic preparations. All of the 20 asymmetric hybrids could be classified into two types

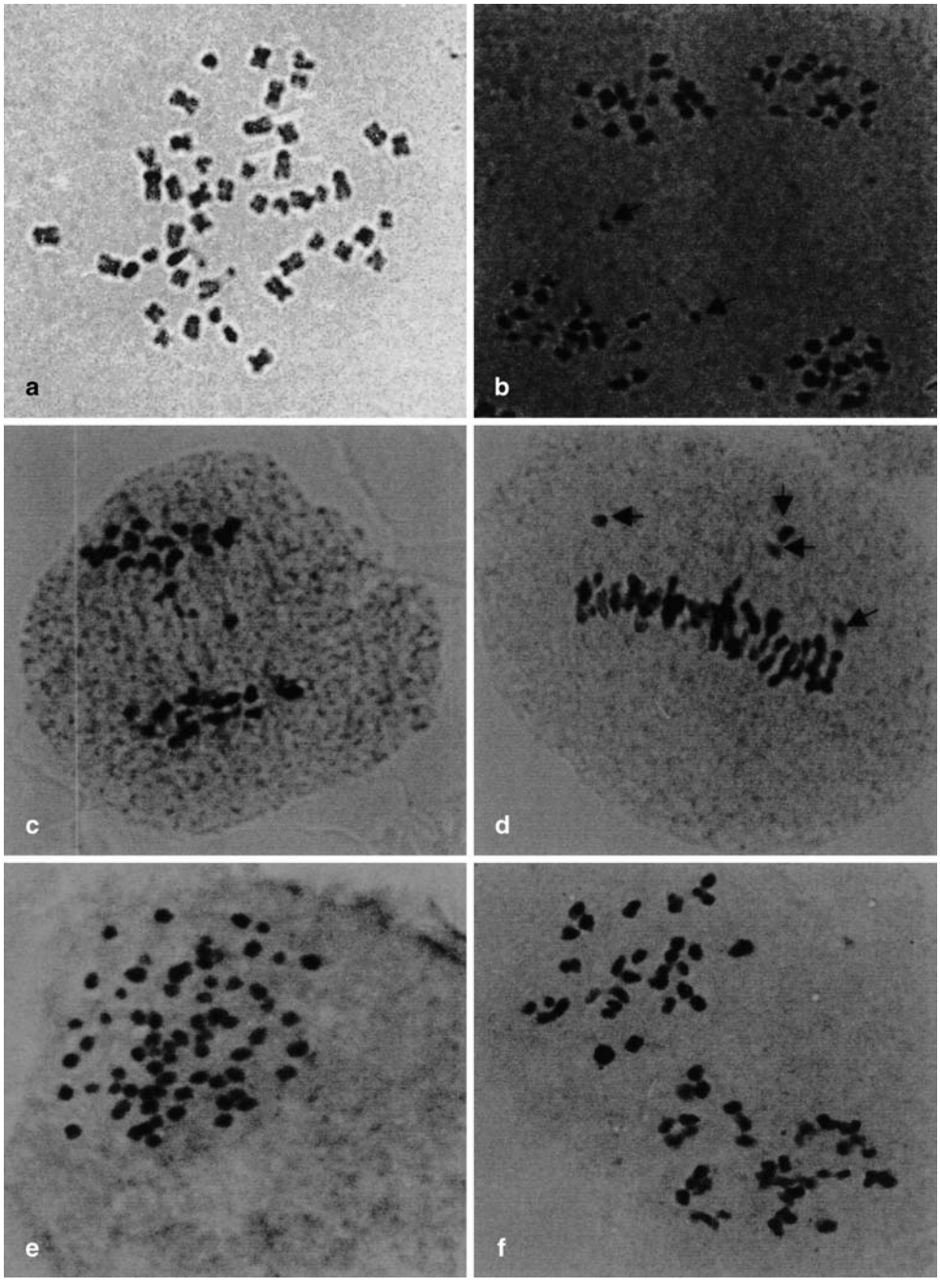

Fig. 6 Cytological observation in F_1 plants. (**a**) Mitotic chromosomes of hybrid no. 4 with 2n = 46. (**b**)–(**f**) Meiosis in some F_1 plants. (**b**) Anaphase of hybrid no. 18 (2n = 38) with unequal distribution and two lagging chromosomes (*arrows*). (**c**) Anaphase of hybrid no. 9 (2n = 38) with some bridge-fragments. (**d**) Metaphase of hybrid no. 5 (2n = 42), 19 bivalents plus 4 unpaired chromosomes (*arrows*). (**e**) Metaphase/anaphase of hybrid no. 1 (2n = 69). (**f**) Anaphase of hybrid no. 1 (2n = 69) with unequal distribution

based on their somatic chromosome number. Type 1 involved nine hybrids which had 38 chromosomes, corresponding to the number of *B. napus* (Table 2). Type 2 had different additional chromosomes, ranging from 40 to 78, but less than the sum of the parental chromosomes, *B. napus* has 38 chromosomes, and *C. abyssinica* has 90 chromosomes (Wang and Luo 1998). As shown in Fig. 6a, hybrid no. 4 had 46 chromosomes with eight additional chromosomes. Meiotic observations were made in all of the F_1 plants. Most of the plants of type 1 with 38 chromosomes exhibited regular meiotic pairing (19 II), whereas the pairing configuration in the meiosis of some plants at diakinesis/metaphase was not normal. Unequal distribution and two lagging chromosomes (arrows) in hybrid no. 18 were observed (Fig. 6b), some bridge-fragments were also observed in hybrid no. 9 (Fig. 6c). Among plants of type 2 with more than 38 chromosomes, additional chromosomes from *C. abyssinica* were observed in the meiosis of the hybrids. They exhibited more meiotic abnormalities. Figure 6d shows the metaphase of hybrid no. 5 (2n = 42) with 19 bivalents plus four unpaired chromosomes (arrows). Figure 6e,f shows the metaphase and anaphase of hybrid no. 1 (2n = 69) with an unequal distribution (33:36).

Table 2 Chromosome numbers and erucic acid content in the seed of the 20 asymmetric somatic hybrids and their fusion parents

Hybrid no.	Chromosome number	Erucic acid content (%) mean ± SD)[a]
1	69	50.9 ± 0.9*
2	78	49.2 ± 0.4
3	50	50.5 ± 0.6*
4	46	49.1 ± 0.8
5	42	49.7 ± 0.8
6	50	46.8 ± 2.0
7	40	43.9 ± 3.4
8	40	49.1 ± 0.8
9	38	48.7 ± 1.2
10	38	48.0 ± 1.4
11	38	49.2 ± 0.7
12	38	47.8 ± 0.7
13	48	49.5 ± 1.0
14	48	50.9 ± 0.9*
15	50	51.2 ± 0.8*
16	38	46.0 ± 0.8
17	38	42.6 ± 3.5
18	38	51.1 ± 0.8*
19	38	48.9 ± 1.1
20	38	50.5 ± 0.9*
B. napus cv 'Maplus'[b]	38	47.9 ± 0.7
C. abyssinica cv 'Galactica'[c]	90	55.0 ± 0.7

[a] A significant difference compared with *B. napus* cv 'Maplus' (LSD = 2.3, $P < 0.01$) is shown by an asterisk
[b] Seeds harvested from the protoplast-regenerated plant grown in the greenhouse
[c] Seeds are original from field-grown

Analysis of fatty acid composition

The erucic acid content was measured in seeds obtained from 20 hybrids by self-pollination or backcrosses with *B. napus*. The majority (15 of 20) of the hybrids had erucic acid levels higher than that of *B. napus* (47.9%), with levels ranging between 48.0% and 51.2% (Table 2). For six hybrids (hybrid nos. 1, 3, 14, 15, 18 and 20), the average erucic acid content differed significantly ($P < 0.01$) from that of the control (*B. napus*), ranging from 50.5% to 51.2%. However, the erucic acid content of five hybrids was lower than that of *B. napus*, ranging from 42.6% to 47.8%. Some hybrids were found to have different ratios of the C_{18} unsaturated fatty acids ($C_{18:1}$:$C_{18:2}$:$C_{18:3}$) compared to the recipient (*B. napus*). Some individuals displayed a ratio of 1:2:2 or higher, whereas the ratio of *B. napus* was about 1:1:1 (data not shown), indicating a changed fatty acid profile in the seed of the hybrids.

Discussion

One of the main difficulties in the production of interspecific and intergeneric hybrids is to obtain sufficient fertility of the hybrids. Both the increased chromosome number in the hybrids and the incompatibility of genome recombination may result in disorders in the formation of gametes as well as seeds. In addition, the level of genome incompatibility influences the genetic integration of the desired traits into the cultivated crops. The production of asymmetric somatic hybrids could be helpful to overcome these difficulties. Using UV-irradiation, the genome of the donor may be largely inactivated and only a small portion of intact DNA will be transferred into the recipient. The present investigation supports this assumption. Nine hybrids with 38 chromosomes had a pollen viability between 70% to 90% and seed set attained a value of 12–22. These hybrids had the same chromosome number as the recipient, even though they were confirmed by nuclear DNA content and AFLP analysis. Similar results were also obtained by Hinnisdaels et al. (1992), Parokonny et al. (1994) in *Nicotiana* and by Skarzhinskaya et al. (1996) in the somatic hybridization between *B. napus* and *Lesquerella fendleri*. In the meiotic observation, most of the F_1 plants exhibited regular meiotic pairing (19II), while some of them showed abnormalities. As shown in the Fig. 6b,c, unequal distribution, lagging chromosomes and bridge-fragments were observed, which may result from intergenomic rearrangements or translocations between *B. napus* and *C. abyssinica* because of UV-irradiation of the *C. abyssinica* protoplast. Therefore, this method could recover a highly asymmetric somatic hybrid, which is very important for plant breeding because small segments of DNA from one species incorporated into the genome of another by chromosome translocations are attractive for the stable introduction of new genes with minimum disruption of the host genome (Parokonny et al. 1994). We have found some hybrids (hybrid no. 18 and 20) with 38 chromosomes which contained a higher content of erucic acid and had a higher seed set. These valuable hybrids could be used for further breeding and good agronomical characters might be accomplished through conventional breeding.

Based on flow-cytometric results, the asymmetric hybrids had a nuclear DNA content greater than that of *B. napus* (recipient) but less than the sum of the parental species. This suggests that UV-irradiation could result in the elimination of donor DNA and the transfer of a part of the *C. abyssinica* DNA to *B. napus*. As shown in the study, 11 hybrids with more than 38 chromosomes could be derived from a complete recipient genome (*B. napus*) and a few additional chromosomes from *C. abyssinica*. Additional chromosomes from *C. abyssinica* were observed in the meiosis of the F_1 plants, and their behavior exhibited more meiotic abnormalities. However, using the traditional cytological method is very difficult to verify which chromosome belongs to *C. abyssinica* because of the similarity in size and shape of the chromosomes of the two species. The hybrids with more than 38 chromosomes had a low pollen viability, ranging from 25% to 60%, and a seed set from 1 to 9. The hybrids nos. 1 and 2 had 69 and 78 chromosomes, respectively. Their pollen viability was only from 25% to 28%, and 1–2 seeds per pollinated flower were obtained when pollinated with *B. napus*. The extremely low pollen fertility observed in the hybrids might be due to both meiotic irregularity and segregational abnormalities. As shown in Fig. 6f, some extra univalents led to an unequal distribution at metaphase of meiosis. The agronomical potential of these hybrids is under current evaluation; further attempts to recover progeny with a high erucic acid content in the seed by self-pollination and backcrosses with *B. napus* are in progress.

In this study, AFLP analysis was used to identify asymmetric somatic hybrids and produced results with high polymorphism, good reproducibility and clear distinguishable bands. Some specific bands from the donor's genome were present using AFLP analysis, although some of the genome was lost because of UV-irradiation of the protoplasts. A considerable variation and many new bands in the AFLP DNA pattern among the hybrids obtained were observed (Fig. 3), which also indicated the intergenomic recombination of *B. napus* and *C. abyssinica*. These results reveal that the hybrids have different degrees of asymmetry. If the donor was treated with 0.05 J/cm^2 UV, more bands from the donor were present in the hybrid, which means that more genetic material from *C. abyssinica* was transferred into the recipient. The hybrids with a nuclear DNA content close to the sum of both parental species had very low pollen viability and led to poor seed set. In contrast, with the increase of UV doses ranging from 0.075 J/cm^2 to 0.15 J/cm^2, the amount of donor DNA in the hybrids was decreased. This demonstrated that very limited donor DNA was transferred to the recipient. The hybrids with a nuclear DNA content more similar to the recipient had a higher pollen viability and a higher seed set, which was correlated with the strength of the UV treatment of the donor protoplasts. This indicates that it is possible to get highly asymmetric hybrids by application of a suitable UV dose, just like hybrid nos. 18 and 20 with 38 chromosomes. They exhibited a significantly higher erucic acid content ($P < 0.01$) than *B. napus* (Table 2). This study supports the observation of Forsberg et al. (1998a) that the degree of asymmetry obtained by UV-irradiation is dose-dependent. An effect of radiation dose on the elimination of donor DNA was also reported by Trick et al. (1994) for asymmetric hybrids between *Nicotiana tabacum* and *Nicotiana plumbaginifolia*, and by Gerdemann-Knörck et al. (1995) who transferred disease resistance within the genus *Brassica* through asymmetric somatic hybridization.

Fatty acid analysis of 20 hybrids revealed that the most of them had a higher erucic acid level than *B. napus* (recipient). Because of the limited seeds harvested from the hybrids, only ten single seeds per hybrid were used for the analysis of erucic acid content. Generally, the total erucic acid content of the hybrids and the *B. napus* parent is lower than expected. The main reason is assumed to be the growing conditions in the greenhouse leading to an incomplete or delayed ripening of the seeds which might influence the fatty acid composition of the seed oil. In particular, the content of erucic acid is known to be increased in the course of seed development, reaching its maximum at the late stage of ripening (Wilmer et al. 1996). Furthermore, certain physiological irregularities due to genomic imbalances might lead to the change of fatty acid composition. The rapeseed parent and the hybrids were grown in the same greenhouse, therefore the comparison of the erucic acid content should be acceptable. The erucic acid content of the ten replications per hybrid was very homogeneous resulting in a low standard deviation. However, to verify the results, the analysis of larger seed samples is needed, which will be possible in the next offspring (F_2 plants) when more plants for each hybrid will be available. Six hybrids were significantly higher than the *B. napus* parent, thus demonstrating that high erucic acid rapeseed could be produced through asymmetric somatic hybridization, which could widen the *B. napus* gene pool. The transfer of the desired trait 'erucic acid content' may be accompanied by other agronomically favorable traits, thus improving the variability usable for breeding purposes. At the same time, the genetic system involved in the formation of the erucic acid in *C. abyssinica* is possibly different from that of *B. napus*. Further studies of the F_2 plants will involve the expression of the *fae* 1 gene, which is a candidate gene governing erucic acid content.

In summary, intergeneric asymmetric somatic hybrids were obtained by using UV-irradiated mesophyll protoplasts of *C. abyssinica* prior to fusion with *B. napus* protoplasts. We found that UV-irradiation did improve the efficiency of production of asymmetric somatic hybrids and promote the fertility of the hybrids. Some hybrids with a higher erucic acid content than *B. napus* were recovered.

Acknowledgements We thank J. Gramenz for help with flow cytometry, Dr. B. Hackauf for instruction on AFLP analysis. This research was supported in part by a fellowship from the Biosciences Special Programme of DAAD, and a grant from Fachagentur Nachwachsende Rohstoffe (FNR).

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