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Q. Hu · L. N. Hansen · J. Laursen · C. Dixelius
S. B. Andersen

Intergeneric hybrids between *Brassica napus* and *Orychophragmus violaceus* containing traits of agronomic importance for oilseed rape breeding

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Abstract Protoplast fusions between *Brassica napus* and *Orychophragmus violaceus* for transfer of valuable traits to oilseed rape resulted in 257 somatic hybrid plants. Hybridity was confirmed by morphological, cytological and molecular means. Symmetric fusions gave rise to 131 plants. Fifty eight of these plants had an intermediate morphology and contained nuclear DNA corresponding to the sum of the parental species. All 131 plants were sterile with no pollen grains observed upon flowering. Another 126 plants were derived from asymmetric fusions in which protoplasts of the donor parent *O. violaceus* were irradiated by 100 or 200-Gy X-rays prior to fusion. Morphologically these plants showed a larger variation compared to the plants regenerated from symmetric fusion experiments. In contrast to plants obtained from symmetric fusions, fertile hybrids were recovered among regenerants from the asymmetric fusions. Twenty four of these plants released viable pollen grains and 14 of the determined 17 plants set seeds after either selfing or backcrossing to *B. napus*. Fourteen male-sterile plants were identified with female fertility. This observed male sterility most-likely originated from alloplasmic recombination and would be of great potential for the development of a new cytoplasmic male sterility system. The fatty acid composition of the fertile hybrids and their progenies showed a biased distribution towards the *B. napus* parent, which has a high erucic acid-content type. However, increased levels of palmitic and linoleic acids compared to *B. napus* were found in subsequent generations, as well as a reduced level of erucic acid.

Keywords *Brassica napus* · Fatty acid composition · Male sterility · *Orychophragmus violaceus* · Somatic hybridization

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Q. Hu (✉) · L.N. Hansen · S.B. Andersen
Department of Agricultural Sciences,
The Royal Veterinary and Agricultural University,
40 Thorvaldsensvej, DK-1871 Frederiksberg C, Denmark
e-mail: Qiong.Hu@vbiol.slu.se
Tel.: +46-18-67-32-48, Fax: +46-18-67-32-79

Q. Hu
Institute of Oil Crops, Chinese Academy of Agricultural Sciences,
430062 Wuhan, P.R. China

J. Laursen
Department of Mathematics and Physics,
The Royal Veterinary and Agricultural University,
40 Thorvaldsensvej, DK-1871 Frederiksberg C, Denmark

C. Dixelius
Department of Plant Biology,
Swedish University of Agricultural Sciences, P.O. Box 7080,
750 07 Uppsala, Sweden

Present address:
Q. Hu, Department of Plant Biology,
Swedish University of Agricultural Sciences P.O. Box 7080,
75007 Uppsala, Sweden

Introduction

Orychophragmus violaceus (L.) O. E. Schulz [syn. *Moricandia sonchifolia* (Bunge) Hook Fil.] is a member of the *Brassiceae* tribe. This species is cultivated as an ornamental plant in China and its wild forms occur both in China and Korea (Luo et al. 1994). Superior oil quality, characterized through the high content of linoleic (53.7%) and palmitic (14.3%) acids, and the low content of linolenic (4.76%) and erucic (0.94%) acids, together with good yield components such as many branches and large seeds, makes it a potential genetic resource for *Brassica* oilseed crop improvement (Luo et al. 1994). Hence, hybridizations between *O. violaceus* and *Brassica napus* for transfer of these traits into oilseed rape have been attempted by sexual means (Li et al. 1995, 1998; Qu et al. 1996; Séguin-Swartz et al. 2000). Sexual incompatibility barriers, reduced fertility and low recombination frequency between the two genomes have unfortunately restricted the evaluation and utilization of the limited hybrid plants produced.

As an alternative approach for combining genetic information, protoplast fusion can be applied for the transfer of traits between distantly related species. This ap-

proach has been rather successful within the *Brassicaceae* family (Waara and Glimelius 1995; Glimelius 1999). A wide range of species in various genera and tribes have taken part in fusion experiments, thus valuable traits encoding for disease resistance (Sjödin and Glimelius 1989; Gerdemann-Knörck et al. 1995; Hansen and Earle 1995, 1997; Sigareva and Earle 1999), fatty acid composition (Hansen and Earle 1994; Heath and Earle 1995; Fahleson et al. 1994), metal hyperaccumulation (Brewer et al. 1999) and cytoplasmic male sterility (Pelletier et al. 1983, 1986; Cardi and Earle 1997) have in this way been transferred across sexual incompatible barriers.

Besides combining two complete genomes by symmetric fusion, asymmetric fusion techniques can be applied for the transfer of only a limited amount of genetic material from a donor species to a recipient. To achieve fragmentation of donor DNA and enhancement of the subsequent integration into the recipient genome, donor protoplasts are in most cases irradiated to induce double-strand DNA breaks, prior to the protoplast fusion step (Dudits et al. 1980; Somers et al. 1986; Forsberg et al. 1998). The most-favourable outcome of this type of limited DNA transfer is the improved fertility of the hybrid plants (Hinnisdaels et al. 1994).

In this study, both symmetric and asymmetric somatic hybrids between *B. napus* and *O. violaceus* were produced with the aim to improve fatty acid content in *B. napus*. Fertile plants were, however, only derived from the asymmetric fusion experiments. Variation in fatty acid composition, as well as morphological performances, was found in the hybrid plants and their offspring. In addition, several male-sterile plants with high female fertility were recovered.

Materials and methods

Plant material and protoplast isolation

Three *B. napus* cultivars, Zhong R, Zhong 4 and Zhong 8 (2n = 38), the latter being the most-widely used winter oilseed rape cultivar in China today, and the *O. violaceus* accession O4834 (2n = 24), were kindly provided by Prof. X.Z. Qian, Institute of Oil Crops, CAAS in Wuhan, China. Seeds were surface-sterilized in 70% ethanol for 3 min followed by 10 min in 1.5% sodium hypochlorite, and germinated on LS medium (Linsmaier and Skoog 1965) containing 3% sucrose. The plantlets were grown at 22–25 °C under cool white fluorescent light, 60–80 μE/m^2s, with a 16-h photoperiod. Newly expanded leaves from 3-week-old plants were used for mesophyll protoplast isolation. They were incubated in an enzyme solution on a gyratory shaker (50 rpm) for 18 h in the dark at room temperature. The enzyme solution contained 0.2 M mannitol, 80 mM $CaCl_2$, 2% cellulase RS (Yakult Honsha Co. Ltd.), 1% macerozyme (Yakult Honsha Co. Ltd.), 0.5% Driselase (Fluka Chemicals), pH 5.8, and diluted in 1.5:3.5 (v/v) with the SCM solution (0.5 M sorbitol, 10 mM $CaCl_2$ and 5 mM MES, pH 5.8).

Pretreatment, fusion and regeneration

In the symmetric protoplast fusion experiments, protoplasts of *O. violaceus* were pre-treated with 3 mM iodoacetate for 30 min to prevent division of unfused protoplasts. No pre-treatment of the *B. napus* protoplasts was applied since the *B. napus* cultivars used had poor regenerability when cultured on regeneration medium lacking silver nitrate (Hu et al. 1999). In the asymmetric somatic fusion experiments, where silver nitrate was added to the regeneration medium, *B. napus* protoplasts were treated by iodoacetate instead of the *O. violaceus* accession. Protoplasts of *O. violaceus* were subjected to X-irradiation at doses of 100 and 200 Gy to fragment its genome. Irradiation was carried out using a Philips compact 400 Hz generator (PW1830/40). Approximately 0.5 ml of densely sedimented protoplast suspension was irradiated at 45 KV and 50 mA at a distance of 20 cm to the tube focus. The protoplasts were purified, fused, and cultured as described by Hansen (1998). Protoplasts were cultured on a series of media (B, C, E, F) according to Pelletier et al. (1983) using a feeder cell system with *B. napus* cells (Walters and Earle 1990). To enhance development and regeneration of asymmetric fusion products, 30 μM of silver nitrate was supplemented to the E medium.

Morphology, ploidy levels, pollen viability and fatty acid analysis

Regenerated plants were characterized according to their morphological features in the greenhouse. Ploidy level was determined by flow cytometry as described previously (Hansen 1998). Fertility was based on pollen viability and seed set. Aceto-carmine (1%) was used to stain pollen for the evaluation of pollen viability microscopically. Hybrid plants without pollen release were pollinated by *B. napus*. Those plants with pollen release were crossed to *B. napus* reciprocally and self-pollinated. Fatty acids were extracted and the composition was analysed by gas chromatography as described by Thies (1971).

PCR analysis

Based on morpologic characterization of the regenerants, putative hybrid plants were investigated by random amplified polymorphic (RAPD-DNA) markers. Plant DNA was isolated from young leaves according to Hu and Quiros (1991). RAPD analysis using ten-base oligonucleotide primers was performed as described by Hansen (1998). More than 100 random primers (Operon Technologies, Inc, USA) were tested and two (P37, 5′-CGCCAGGAGC-3′, and OPR-11, 5′-GTAGCCGTCT-3′) were selected based on clear polymorphism and used to assess the obtained plants.

Mitochondrial DNA analysis

The mtDNA analysis was performed on total DNA (Landgren and Glimelius 1990) isolated from parental species and hybrid plants. The DNA was digested with *Bam*HI, separated by 0.7% agarose-gel electrophoresis and transformed to Hybond-N^+ filters (Amersham Pharmacia Biotech) according to the manufacturer's instruction. Probes for the mitochondrial genes *coxII* and *atp9* were PCR amplified from *Arabidopsis thaliana* DNA using primer pairs designed according to the complete *A. thaliana* mtDNA sequences (Unseld et al. 1997). Labelling, hybridization and filter washings were performed as described by Schröder-Pontoppidan et al. (1999).

Results

Symmetric hybridization

Plant regeneration and morphology

In total, 4,766 calli were isolated from the three fusion combinations between *B. napus* and *O. violaceus*. Shoot regeneration efficiency varied from 3.3 to 35.8% and 131 plants from 114 calli were established in the greenhouse (Table 1).

Table 1 Irradiation dosage, number of calli, regenerating calli, regeneration efficiency and plant production in protoplast fusions between *B. napus* and *O. violaceus*

B. napus genotype used in fusion	X-ray dosage (Gy)	No. of calli	No. of regenerating calli	Regeneration efficiency (%)	No. of plants	Fertile plants[a]
Zhong 8	0	1,697	480	28.3	46	0
Zhong 4	0	2,113	69	3.3	16	0
Zhong R	0	956	342	35.8	69	0
Zhong 8	100	764	164	21.5	98	29
Zhong 8	200	854	96	11.2	28	8

[a] Includes plants with aceto-carmine stainable pollen and plants that set seeds

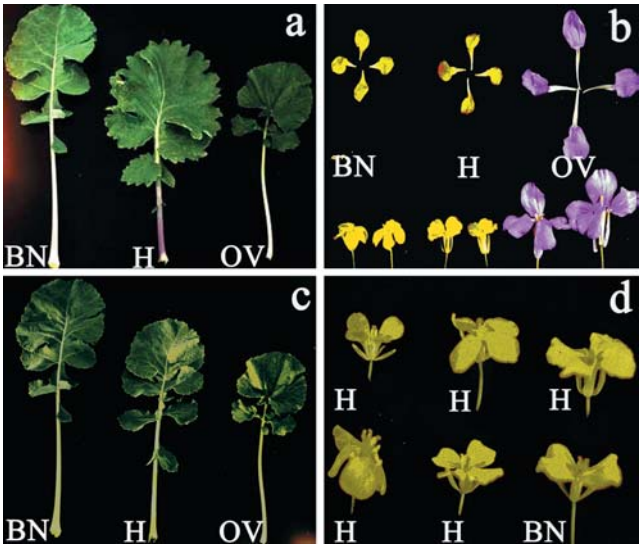

Fig. 1a–d Morphology of *B. napus* (+) *O. violaceus* hybrids. **a** Basal leaf of parents and a hybrid from symmetric fusion. **b** Flowers and flower petals of parents and a hybrid from symmetric fusion. **c** Basal leaf of parents and a hybrid from asymmetric fusion. **d** Variable flower morphology of hybrids derived from asymmetric fusion. *BN*: *B. napus*; *H*: Hybrid; *OV*: *O. violaceus*

A majority (85 plants) possessed a morphology intermediate to the parental species. Lobation of the basal leaf margins resembled that of *B. napus* but with a semi-spheric top leaflet typical of *O. violaceus* (Fig. 1a). Flowers and buds were intermediate in size to the parentals, slightly larger than *B. napus* but much smaller than *O. violaceus* (Fig. 1b). Hybrid plants bolted in a similar manner as *B. napus* with one main stem, whereas *O. violaceus* had several slender and shorter axillary stems. More branches, especially secondary, tertiary and even quaternary branches, were also typical for these plants. However, extensive variation in leaf colour, axillary shooting, bolting time and petal colour was observed among these plants.

DNA content, fertility and RAPD analysis

Ninety two putative hybrid plants were selected for nuclear DNA measurement by flow cytometry. The *B. napus* parental cultivars had a nuclear DNA content of 2.2 ± 0.03 pg/cell while *O. violaceus* had a DNA content of 2.5 ± 0.02 pg/cell. All the plants contained a nuclear DNA higher than either of the parental lines, ranging from 3.7 to 7.1 pg/cell. The plant population was further classified into four groups depending on their nuclear DNA content. Fifty plants had a nuclear DNA content corresponding to the sum of parents (Group 1) and eight plants had a somewhat lower DNA content (Group 2). These two groups accounted for 63% of the hybrid population. The third group consisted of 24 plants with a nuclear DNA content higher than the sum of the parental lines. Twelve plants in this group might have had a triplicate origin since their nuclear DNA content was close to the total of three cells. Ten plants that showed a mixoploid nature were classified in the fourth group.

None of the 131 plants derived from the symmetric fusion experiments were fertile. Their flowers had six non-elongated stamens and anthers that were pale at the beginning of flowering but turned rapidly brownish. No release of pollen was observed from opened flowers. Microscopic studies revealed only a few stainable microspores in flower buds prior to flowering. Backcrosses with the *B. napus* parent as the pollinator of more than 1,000 flower buds did not give rise to any seeds. A hampered ovary development was observed 5 to 7 days after pollination. Attempts to culture ovaries and ovules in vitro did not result in the development of any embryos or plants.

RAPD analysis was performed only on plants regenerated from the fusion of Zhong 8 and O4834, because Zhong 8 is the most interesting cultivar in China from a breeding point of view. All the 40 analysed plants had at least one band from each of the parental species and the DNA banding pattern of most of the plants resulted from the addition of the parental lines (Fig. 2a).

Asymmetric hybridization

Regeneration of plants and morphology

Asymmetric protoplast fusions were made using *B. napus* cv Zhong 8 and O4834 as the parental lines. Two fusion experiments applying irradiation doses of 100 and 200 Gy (hereafter referred to as “Fusion 1” and “Fusion 2”, respectively), were performed. In total, 1,618 calli were isolated and 260 of them initiated shoot formation. Plant regeneration frequency was lower compared to

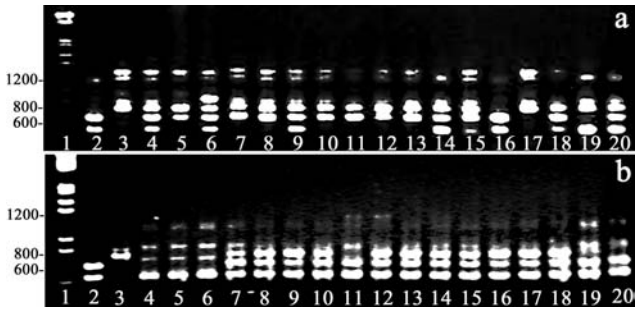

Fig. 2a, b RAPD pattern of somatic hybrids amplified using P37 (5′-CGCCAGGAGC-3′). **a** Hybrids from symmetric fusion. *Lane 1*: Lambda DNA/*Eco*RI + *Hin*dIII marker. *Lanes 2 and 16*: Zhong 8. *Lanes 3 and 17*: O4834. *Lanes 4–15*, *18–20*: hybrids. **b** Hybrids from asymmetric fusion. *Lane 1*: Lambda DNA/*Eco*RI+*Hin*dIII marker. *Lane 2*: Zhong 8. *Lane 3*: O4834. *Lanes 4–20*: hybrids

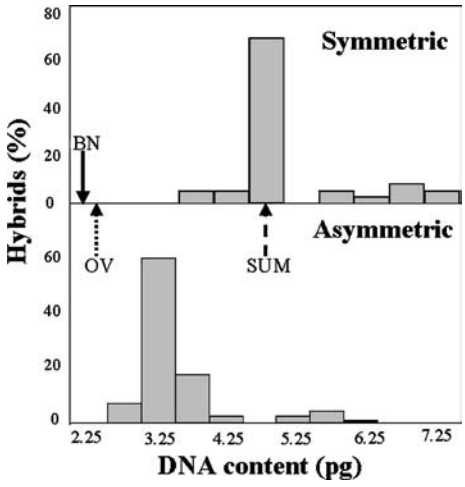

Fig. 3 Comparison of nuclear DNA content of somatic hybrids obtained from symmetric (*upper*) and asymmetric (*lower*) fusions. *BN*: *B. napus*; *OV*: *O. violaceus*; *SUM*: sum of the parentals

symmetric fusions of the same parental lines, especially in the experiment where a higher X-irradiation dosage was applied (Table 1). As many as 150 plantlets originating from 23 calli were found to contain more nuclear DNA compared to *B. napus*. From these plantlets, 126 derived from 15 different calli were successfully established in the greenhouse. A large amount of plants could be obtained from one callus, e.g. 87 were developed from callus 2 in "Fusion 1" (and more could be developed with continuous culture). Compared with the plants originating from the symmetric fusion experiments, most plants from the asymmetric fusions had an overall appearance more inclined to *B. napus*, such as plant habit, leaf and flower morphology (Fig. 1c and d). The intermediate morphology of these plants could be observed by lobation of the basal leaf margins, leaf colour, as well as leaf-edge serrates. However, more distinct morphological variation was observed among these plants compared to those from symmetric fusions, concerning several plant parts such as leaf-edge serration and color, flower size and petal shape, branch number and angle.

Nuclear DNA content and RAPD analysis

From Fusion 1, 101 plantlets had a nuclear DNA content higher than Zhong 8, ranging from 2.9 to 6.2 pg/cell. Four calli regenerated plants with a nuclear DNA content higher than the sum of the parents. The remaining ten calli gave rise to plants with a nuclear DNA content lower than the sum of parents. A range of 2.8–5.2 pg/cell of nuclear DNA content was measured among 14 plants derived from six calli in "Fusion 2". Two plants, originating from one callus, both contained a nuclear DNA content higher than the sum of the parental species. The remaining five calli regenerated 12 plants with a nuclear DNA content less than the sum of parents. Seven out of the analysed 115 plants were mixoploids. The difference in the average nuclear DNA contents between plants from the two asymmetric fusion experiments was not significant (3.6 vs 3.5 pg/cell). In contrast, compared with plants from the symmetric fusion of the same parental combination, plants regenerated from the asymmetric fusions had a significantly lower content of nuclear DNA ($F = 58.17$, $P = 0.0001$). These plants had an average nuclear DNA content of 3.5 ± 0.07 pg/cell, whereas the average nuclear DNA content of plants derived from symmetric fusions was 5.1 ± 0.12 pg/cell (Fig. 3).

RAPD analysis was performed with plants regenerated from both fusion experiments. Fifty hybrids were analysed using primers P37 and OPR-11, including all the fertile ones. Forty six plants had at least one extra fragment from each of the parental species (Fig. 2b) whereas four plants had a RAPD pattern lacking at least one band from the parents, indicating the possible elimination or/and rearrangement of the two combined genomes.

Recovery of fertile hybrids

Twenty four hybrid plants derived from five calli released stainable pollen upon flowering. These plants had stamens elongated to various extents, from half to three quarters of normal length, and produced half the amount of pollen grains compared to the fertile *B. napus* parent. The remaining hybrid plants had a reduced size or number of stamens and did not release any pollen grains. Among the plants which released viable pollen grains, 17 were determined and 14 were identified to be fertile and set seeds after self-pollination or/and backcrossing to the *B. napus* parent (Table 2). Plants 13-1 and 13-2 were able to fertilize *B. napus* ovules and developed seeds, although no seeds were harvested after self-pollination or open-pollination. Three plants only set seeds by backcrossing with *B. napus* pollen (31-3 and 31-7) or open-pollination (31-8). There was no correlation between any two of the three parameters – nuclear DNA content, pollen viability and seed set.

From the hybrid plants that did not release pollen grains, 14 were identified as male-sterile but with female fertility (Table 2). Seeds were obtained from these plants

Table 2 Nuclear DNA content, pollen viability and fertility of asymmetric hybrids

Plant[a]		Nuclear DNA content (pg/cell)	Pollen viability (%)	Fertility[b]		
				Self pollination		Pollinated by *B. napus*
				Open flower	Bud	
Male-fertile hybrids	13-1	2.89	71 ± 2	0	0	0
	13-2	2.92	71 ± 3	0	0	0
	31-3	3.04	65 ± 6	0	0	33.0
	31-4	3.10	73 ± 2	11.2	16.3	42.7
	31-6	2.93	47 ± 3	0	18.5	nd
	31-7	3.12	71 ± 7	0	0	24.2
	31-8	3.16	49 ± 6	0	0	nd
	31-9	3.11	63 ± 5	13.2	nd[c]	21.6
	31-11	3.12	76 ± 7	11.5	19.5	52.0
	31-14	3.08	59 ± 7	9.4	34.4	73.9
	31-20	nd	74 ± 3	6.0	13.7	35.1
	31-22	nd	77 ± 5	6.1	11.6	34.1
	8-3	2.81	89 ± 4	0	nd	9.5
Male-sterile hybrids	31-1	3.16	0	0	0	17.8
	31-2	3.08	0	0	0	48.7
	31-5	3.03	0	0	0	10.5
	31-7	3.12	0	0	0	21.9
	31-10	3.04	0	0	0	31.7
	34	3.22	0	0	0	30.2
	23-1	3.45	0	0	0	16.0
	23-2	3.46	0	0	0	10.5
	23-4	3.42	0	0	0	6.0
	23-8	3.39	0	0	0	8.0

[a] All the plants were derived from Fusion 1 (X-irradiation does of 100 Gy), except 8-3 that derived from Fusion 2 (X-irradiation dose of 200 Gy)

[b] Seed per silique calculated as % of *B. napus*

[c] nd: not determined

after fertilization by *B. napus* pollen. The female fertility ranged from 6.0 to 48.7% compared to *B. napus*, which means that 1–8 seeds per silique were harvested after being pollinated. Five of these male-sterile hybrids were derived from the same callus (callus 31) as the ten male-fertile hybrids. This fertility segregation was not observed among eight male-sterile plants derived from callus no. 23.

MtDNA analysis of progeny from asymmetric hybrids

Twelve plants derived from three somatic hybrids, 31-2, 31-9 and 8-3, either by backcrossing (the male-sterile plant 31-2) or self pollination (male-fertile plants 31-9 and 8-3) were analysed for mitochondrial DNA composition. Five plants exhibited an *O. violaceus* restriction pattern for both probes (Fig. 4). The *atp9* probe displayed an *O. violaceus* restriction pattern for the ten plants (Fig. 4b) whereas the *coxII* probe visualized an *O. violaceus* specific pattern in only five plants (Fig. 4a). Five out of six plants derived from somatic hybrid 8-3 had an *O. violaceus* restriction pattern when probed with the *atp9* gene, but a *B. napus* pattern with the *coxII* gene.

Fatty acid composition of seeds from asymmetric hybrids and their offspring

The content of seven major fatty acids, (palmitic, stearic, oleic, linoleic, linolenic, eicosanoic and erucic acids)

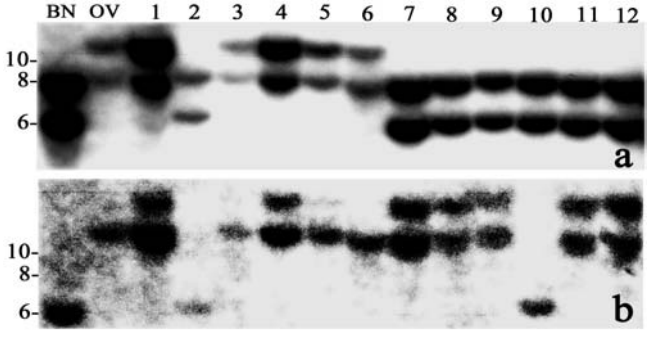

Fig. 4a, b Mitochondrial DNA composition of the progeny from three somatic hybrids. *BN*: *B. napus*; *OV*: *O. violaceus*; **a** Probed with *coxII*. **b** Probed with *atp9*. Plant nos. 1–3, 4–6, 7–12 are derived from three different hybrids respectively

were analyzed in the asymmetric hybrids by a "half-seed" method and compared to the parental species. Both self-pollinated and backcrossed progeny showed the contents of all major fatty acids to be biased towards the *B. napus* parent. However, individual seeds with fatty acid profiles significantly divergent from *B. napus* were also obtained. Among 171 analysed seeds of three progeny populations (S_1, BC_1 or BC_2) of 14 asymmetric hybrids, 34 had a palmitic acid content higher than 4%. The highest content of palmitic acid reached 5.9% in seed harvested after the selfing of hybrid 2–10, whereas *B. napus* Zhong 8 contained only 3.1%. All 11 seeds from hybrid

2–10 contained much-higher palmitic acid than *B. napus*, ranging from 4.2 to 5.9% with an average of 4.9%. A linoleic acid content above 19% was detected in four of the seeds harvested from the asymmetric hybrids, which is significantly higher than found in the *B. napus* parent (13.7%). Seeds containing an erucic acid level lower than the *B. napus* parent were found both in BC_1 and S_1 plants. Twelve seeds (4 BC_1 and 8 S_1) had an erucic acid level less than 40%, with the lowest content in one seed being 33.2%. The 12 seeds harvested from the *B. napus* parent Zhong8, grown under the same conditions as those hybrids, had an erucic acid content ranging from 45.4 to 52.7% with an average of 49.2%.

Discussion

Protoplasts from *O. violaceus* have a high capability to regenerate plants (Hu et al. 1999). This character mostlikely enhanced the rather high plant regeneration frequencies (3.3–35.8%) obtained from the fusion experiments in the present study. This result can be compared with attempts to sexually cross *B. napus* with *O. violaceus* (Li et al. 1995). Only three *B. napus* genotypes gave rise to offspring in that case, while four other *B. napus* genotypes were sexually incompatible with *O. violaceus* even though embryo culture was applied. All plants derived from the symmetric fusions between the two species were completely sterile, which is opposite to the situation found from the sexual crosses (Li et al. 1995). The genetic constitution of the two kinds of hybrids is very different. The sexual hybrids were triploid with 31 chromosomes. According to cytological and morphological observations, less than 5% of the plants in the subsequent selfed F_3 and F_4 generations were classified as a hybrid type. The remaining offspring showed a *B. napus* phenotype, presumably due to complete genome elimination, which also accounted for the high fertility of the primary hybrid plants (Li et al. 1995). In contrast, most plants from symmetric fusions obtained in this study contained a nuclear DNA content corresponding to the sum of parents, indicating that a complete set of *B. napus* and *O. violaceus* chromosomes were retained. If genome elimination had occurred in these hybrid plants, some of them should have released viable pollen grains of parental type and set seed.

One way to overcome problems with hybrid sterility is to produce asymmetric hybrids. The fertility of intergeneric species combinations within the *Brassicaceae* has in other cases been improved by asymmetric hybridization. In the combination between *B. napus* and *Lesquerella fendleri*, 38% of those plants derived from asymmetric fusions could be successfully selfed (Skarzinskaya et al. 1996). A similar positive correlation between the degree of asymmetry and the seed set after selfing-results were found in asymmetric *B. napus* and *A. thaliana* hybrids (Forsberg et al. 1998). A positive effect of asymmetry was also found in the present case where all fertile plants were derived from asymmetric fusions. The fertility of the asymmetric hybrids (6.0–34.4% compared to *B. napus*) was in some cases comparable to that obtained after sexual crosses (18.5%). The high fertility of the sexual hybrids was presumably attributed to genome separation, which means the elimination of a complete genome from one of the parents during meiosis (accordingly, only gametes of one parental type were produced). The different competitive capacity of the parental gametes may explain the fact that most of the plants were of a *B. napus* type and a few were of hybrid type in the offspring of subsequent generations (Li et al. 1995). According to the nuclear DNA-content analysis, the asymmetric hybrids produced in this study had 25–50% extra DNA, presumably of the *O. violaceus* type present in the *B. napus* background. A closer look at the genome content using genomic in situ hybridization (Snowdon et al. 1997) would help to clarify the extent of the *O. violaceus* DNA retained in this material since no *O. violaceus*-specific DNA markers exist.

As found in progenies of symmetric hybrids between *B. napus* and *Sinapis arvensis* (Hu et al. 2002), the content of the major fatty acids in seeds harvested from the primary asymmetric hybrids and their progenies all showed distributions biased towards the *B. napus* parent. Seeds derived from somatic hybrids containing particular fatty acids significantly divergent from the *B. napus* fusion parent were found, such as those with higher palmitic acid and lower erucic acid. The altered fatty acid composition may be caused by the functioning of the genetic factors determining the high content of palmitic and the low content of erucic acid characters in the donor parent *O. violaceus*, which were incorporated into the hybrids.

Somatic hybridization is furthermore a valuable tool for the induction of novel cytoplasmic male sterility (CMS) systems. In *Brassica* crops, *tour* CMS (Stiewe and Röbbelen 1994), *Trachy* CMS (Kirti et al. 1995) and *Mori* CMS (Prakash et al. 1998) were derived from somatic hybridizations. The selection of male-fertile hybrids synchronously has resulted in the transfer of fertility restoration factors from related *Brassica* species (Prakash et al. 1998). Enrichment of genetic variation at the cytoplasmic level is highly desirable for developing new and improved CMS systems, and to reduce the risk of epidemic diseases associated with cytoplasm such as the southern corn leaf blight in the T-CMS of maize (Levings 1990). The male-sterile asymmetric hybrids between *B. napus* and *O. violaceus* obtained in the present study are a potential source of new CMS material. There have not been any reports on the occurrence of male sterility from sexual hybrids between *O. violaceus* and *Brassica* species. Incorporation of the cytoplasmic genome of *O. violaceus* and the interaction of *O. violaceus* mtDNA with the *B. napus* nuclear genome in the hybrids may account for the induction of male sterility in these plants.

It can be concluded that asymmetric hybridization is an efficient approach to introduce both the nuclear and cytoplamic genomes of *O. violaceus* into *B. napus* for the diversification of the oilseed rape gene pool. Besides agronomically important characters, such as enhanced branching and big seeds, the improved fatty acid compo-

sition and the transfer of cytoplasmic DNA, which in this case is a potential source for the development of a new CMS system, are characters of great importance for further oilseed rape breeding.

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