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Fertility and chromosome stability in *Brassica napus* resynthesised by protoplast fusion

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Summary. Fertile somatic hybrids between *Brassica campestris* and *B. oleracea* have been produced by protoplast fusion. Fusion products were identified by their intermediate protoplast morphology. Heterokaryons were isolated either with micropipettes using a micromanipulator or by flow sorting. About 2% of the obtained calli differentiated to shoots. Of the shoots obtained from manually selected heterokaryons, 100% were true hybrids as confirmed by isozyme analysis while 87% of the flow sorted ones showed a hybrid pattern. Ploidy level of the hybrid plants was determined by chromosome counting and relative DNA-content analysis. The sum of the chromosome number (38) from the two fusion partners were found in 30% of the hybrids; 9% had fewer and 61% had more chromosomes. Pollen viability and seed set varied with ploidy level. Compared to natural *B. napus*, a pollen viability of 52%–93% and a fertility of 1%–40% was found for the somatic hybrids with normal chromosome number. Restriction enzyme analysis of chloroplast-DNA showed that either *B. campestris* or *B. oleracea* chloroplasts were present in the somatic hybrid plants. Of 11 hybrid plants 5 had the campestris and 6 had the oleracea type (1 : 1 ratio).

Key words: *Brassiceae* – Protoplast fusion – Flow sorting – Fertility – Chloroplast-DNA

Introduction

Sexual incompatibility barriers between species can be bypassed by somatic hybridization. The technique gives new possibilities for increasing the genetic variation in our crop plants by facilitating gene flow from one species to another (Schieder and Vasil 1980; Evans and Flick 1983; Harms 1983). It also provides possibilities for rearrangements of the cytoplasmic genomes (Pelletier et al. 1983), and is therefore of great potential value for the plant breeder.

The practical importance of somatic hybridization needs to be evaluated. Even if an interspecific hybridization, somatic or sexual, is successful, the utilization of the obtained hybrids can be problematic. Interspecific hybridizations are often connected with chromosome instability and lack of fertility. Somatic hybrids are produced between diploid cells, which should facilitate meiotic balance. However, chromosome numbers are often quite variable (Evans 1984), causing disturbances in the meiosis and decreased fertility of the material. Fertility of the hybrid is of utmost importance if it is to be used as bridging material for transferring specific traits or function as a new allopolyploid. Several interspecific somatic hybrids in different genera obtained to date have been analysed for fertility. Some show none or very low self-fertility (Hoffman and Adachi 1981; Schenck and Röbbelen 1982; Gleddie et al. 1986). Other somatic hybrids show different degrees of fertility (Schieder 1980) even though their corresponding sexual hybrid was sterile (*Lycopersicon esculentum* × *L. peruvianum*, Kinsara et al. 1986). Whether the problems with low fertility and chromosome instability is different in somatic hybrids and sexual hybrids needs further evaluation.

In the present study, we have resynthesed rapeseed (*Brassica napus*) through somatic hybridization between *B. oleracea* and *B. campestris*. Even though somatic hybrids between these species had been obtained (Schenck and Röbbelen 1982; Robertson et al. 1987; Sundberg and Glimelius 1986; Terada et al. 1987) fertility of the prior hybrids had not been studied. Since resynthesis of rapeseed has been made by sexual crossings

(Olsson 1960; Inomata 1980; Akbar 1987a, b), an evaluation and comparison of different traits connected to fertility was possible between the sexual and the somatic hybrids here produced.

Materials and methods

Plant material

For the resynthesis of rapeseed, *B. napus,* either of four varieties of *B. oleracea* L. were used as one of the fusion partners: (1) *B. oleracea* ssp. *botrytis,* var. asparagoides, cv Green Mountain; (2) *B. oleracea* ssp. acephala, var. acephala, "Grönkål"; (3) *B. oleracea* ssp. acephala, var. acephala, "Höstkål" and (4) *B. oleracea* ssp. botrytis, var. botrytis, "Blomkål". The other fusion partner used was either of four varieties of *B. campestris* L. ssp. *oleifera,* (1) cv Emma, (2) Sv 03242, (3) Sv 01082 and (4) Sv 03261. In the fertility tests, the somatic hybrids were crossed with *B. napus* L. ssp. *oleifera* cv Hanna. All seeds were kindly provided by W. Weibull AB and Svalöf AB, Sweden.

Protoplast isolation and fusion

Protoplast isolation was performed according to Glimelius (1984). From one of the fusion partners 4-day-old hypocotyls were used and from the other partner leaves of a 3-week-old plant grown in vitro were used to produce morphologically different protoplasts.

In the experiments where the fusion products were enriched by flow sorting, the hypocotyl protoplasts were stained with carboxyfluorescein diacetate (12 µg/ml) during enzyme treatment. The fusion method (PEG) was as described by Glimelius et al. (1978) with modifications (Sundberg and Glimelius 1986). Directly after fusion, the protoplasts were incubated in 8p medium (Kao and Michayluk 1975) modified as described by Glimelius et al. (1986).

Selection and culture of hybrid cells

Two selection methods were used. From 21 experiments, the heterokaryons were isolated with micropipettes handled with a micromanipulator. The protoplasts were transferred to a multivial dish, where 100 heterokaryons were cultured in 10 µl modified 8p medium according to Sundberg and Glimelius (1986). About 500 heterokaryons were isolated in each experiment. In the other selection procedure, heterokaryons from 15 experiments were sorted in a FACS III fluorescence activated flow sorter (Becton Dickinson) according to the method of Glimelius et al. (1986). In each experiment, 2,000 to 20,000 protoplasts were sorted into a multivial dish where each vial contained 150 µl modified 8p medium. Approximately 2,000 to 3,000 heterokaryons were sorted into each vial. The culture conditions were according to Glimelius (1984) with the following modifications. After 2–5 days, or when the first divisions were obtained, the cells were diluted with new culture medium (3× the original volume). Regeneration of calli and differentiation into shoots were performed as developed by Glimelius (1984).

Confirmation of hybrid character by isozyme analysis

Isozyme analysis was performed using leaf tissue of the parental and the hybrid plants, according to the methods described by Sundberg and Glimelius (1986).

Chromosome and ploidy level analysis

From each hybrid plant, 5–10 cells from 2–3 root tips were analysed for chromosome number. For contraction of the chromosomes, the root tips were pretreated with 0.015 M 8-hydroxyquinoline solution for 1 h before fixation in absolute ethanol-glacial acetic acid (3 : 1). The root tips were then hydrolyzed in 1 N HCl at 60 °C for 10 min and stained in Feulgen for 30 min. The root tips were squashed in 2% acetoorceine and examined for metaphase cells.

As a complement to chromosome counting, quantitative flow cytometric DNA analysis (Sundberg and Glimelius 1986; Fahleson et al. 1987) of the hybrid plants were performed. Protoplasts from 2–3 leaves of each hybrid plant were isolated as described by Glimelius (1984) and the cell nuclei of the protoplasts were prepared and stained with propidium iodide according to Vindeløv et al. (1983). To standardize the DNA-axis equally in all experiments two reference standards, human lymphocytes and protoplasts of *B. campestris,* were added to each preparation. Aliquots of the lysate, corresponding to 10^4–10^5 nuclei, were analysed by a FACS-III flow cytometer (Becton-Dickinson), as described by Sundberg and Glimelius (1986) and Fahleson et al. (1987). The DNA-content was determined as the relative PI-fluorescence and expressed as arbitrary units normalized to *B. campestris.*

Analysis of pollen viability

Viability of pollen was determined by applying a method for vital staining of cells using fluorescein diacetate (FDA) as a marker (Heslop-Harrison and Heslop-Harrison 1970). From every hybrid plant, five flowers were collected. The anthers of each flower were immediately suspended in 0.5 ml of a solution containing 75 mg/ml sucrose, 0.05 mg/ml H_2BO_3, 0.07 mg/ml $Ca(NO_3)_2$, 0.1 mg/ml $MgSO_4$, 0.17 mg/ml KH_2PO_4 (I. Kristiansdottir, personal communication) and 1 µg/ml FDA. The number of viable pollen was determined as the number of pollen emitting fluorescence when subjected to UV light with a wavelength of 365 nm.

Analysis of hybrid fertility

For each hybrid plant, approximately 100 mature flowers were self-pollinated, 100 were bud pollinated, 100 mature flowers were pollinated with *B. napus* and 50 flowers were pollinated with *B. campestris.* The number of obtained siliquas were recorded, and the number of placentas/siliqua were counted for 20 siliquas of each hybrid plant. The hybrid fertility was determined as number of obtained seeds/number of produced placentas in the obtained siliquas.

Morphological characterization of the hybrid plants

Plant height of the hybrids was determined at maturity (during seed development). Leaf shape, amount of trichomes and wax on the leaves, thickness of the plant and flower abnormalities were recorded from actively growing hybrid plants.

Extraction of chloroplast-DNA

Chloroplast-DNA was isolated according to a slightly modified method of Palmer (1986) using young greenhouse-grown plants. About 10–15 g leaves were homogenized in 500 ml of buffer A (0.3 M sorbitol, 50 mM Tris-HCl, 20 mM EDTA; pH 8.0) containing 0.1% BSA. The homogenate was filtered through nylon meshes of 50 µm and 20 µm, respectively, and centrifuged at 1,600 g for 10 min. The pellet was resuspended and washed in a total volume of 30 ml of buffer A and centrifuged again (1,600 g, 10 min). The chloroplasts were washed

Table 1. Chromosome number, fertility and chloroplast genotype of the somatic hybrids and their parents

Fusion partners	Hybrid no.	Chromosome no.	Relative DNA content mean ± 0.003 (SEM)	Chloroplast genotype	Pollen viab. %, $n=5$ mean ± SEM	Seed set no. seed/placenta			No. of placentas/siliqua, mean ± SEM $n=20$
						Selfed	× *B. napus*	× *B. campestris*	
Emma × Blomkål	h1	38	2.0	*B. campestris*	38 ± 2.9	0.003	0.013	0.016	35 ± 0.5
Emma × Blomkål	h2	38	1.9	*B. campestris*	52 ± 1.9	0.004	0.047	0.026	36 ± 0.5
Emma × Blomkål	h3	38	2.1	*B. campestris*	68 ± 3.3	0.021	0.050	0.123	23 ± 0.3
Emma × Blomkål	h4	38	2.3	ND	53 ± 16.0	0.096	0.390	0.152	26 ± 0.3
Sv 03261 × Blomkål	h5	38	2.3	*B. campestris*	70 ± 2.6	0.115	0.046	0.034	26 ± 0.6
Sv 03261 × Blomkål	h6	38	2.3	*B. oleracea*	57 ± 3.2	0.037	0.134	0.107	29 ± 0.4
Sv 03242 × Broccoli	h7	38	2.2	ND	5 ± 3.2	ND	ND	ND	ND
Sv 01082 × Broccoli	h8	35[a]	2.2	*B. campestris*	25 ± 1.5	ND	ND	ND	ND
Sv 01082 × Broccoli	h9	32	2.0	ND	ND	ND	ND	ND	ND
Sv 03242 × Broccoli	h10	68	3.6	ND	no flowers	–[b]	–	–	–
Sv 03242 × Broccoli	h11	57	3.4	ND	no pollen	–	0	0	–
Emma × Blomkål	h12	51	3.6	*B. oleracea*	15 ± 2.5	0.0001	0.120	0.085	32 ± 0.4
Emma × Blomkål	h13	58	3.3	ND	19 ± 3.0	0	0.017	0	36 ± 0.5
Emma × Blomkål	h14	56	3.4	*B. oleracea*	15 ± 2.7	0.002	0.031	0.004	20 ± 0.5
Emma × Blomkål	h15	53	3.8	ND	no pollen	–	ND	ND	ND
Emma × Blomkål	h16	52	3.5	*B. oleracea*	32 ± 4.9	0.001	0.117	0.039	29 ± 0.4
Emma × Broccoli	h17	48	3.0	*B. oleracea*	6 ± 1.8	0.0003	0.068	0	22 ± 1.1
Emma × Blomkål	h18	50[a]	3.6	ND	14 ± 6.0	0	0.064	0.0004	27 ± 0.3
Sv 03242 × Broccoli	h19	58	3.6	ND	40 ± 8.2	ND	ND	ND	ND
Sv 03242 × Broccoli	h20	50[a]	3.3	ND	11 ± 4.0	0	0.005	0	23 ± 0.3
Sv 03242 × Broccoli	h21	86[a]	7.2	ND	ND	ND	ND	ND	ND
Emma × Höstkål	h22	55	2.4/3.7	ND	0	0	0	0	–
Sv 03261 × Blomkål	h23	78[a]	2.4/4.8	*B. oleracea*	12 ± 3.3	0	0	0	–
Parental material and normal rapeseed used as control plant									
B. napus cv Hanna		38	2.2	ND	75 ± 3.9	0.287	ND	ND	27 ± 0.5
B. oleracea cv Blomkål		18		*B. oleracea*	67 ± 4.6	0.327	ND	ND	28 ± 2.2
Broccoli		18	1.2	*B. oleracea*	72 ± 7.4	ND	ND	ND	ND
B. campestris cv Emma		20	1.0	*B. campestris*	75 ± 3.3	ND	ND	ND	ND

[a] Mean ± 2–5; [b] estimation not possible

4 times, resuspended in 9 ml buffer A and further purified on a sucrose gradient consisting of 60%:45%:20% sucrose, all in buffer A. Gradient centrifugation was made at 26,000 rpm for 1 h (Beckman L2, 65 B, sw 41 rotor) and chloroplasts were removed from the interphase between 20% and 45% sucrose. The chloroplasts were diluted with 3 volumes of buffer A and centrifuged at 3,000 g for 5 min. The final pellet was resuspended in 3 ml of buffer A and lysed by adding 0.6 ml buffer B (0.5% SDS, 50 m*M* Tris-HCl, 0.4 M EDTA; pH 8.0) containing 1 mg/ml proteinase K for 15 min at 50 °C. Chloroplast-DNA was purified by phenol/chloroform extractions and DNA was ethanol-precipitated at –20 °C overnight.

Restriction enzyme analysis and gel electrophoresis

Digestion of chloroplast-DNA with restriction enzyme was performed in 20 µl of a reaction mixture by using an excess of enzyme for 2 h at 37 °C. Chloroplast-DNA was digested with *Bam*HI, *Eco*RI, *Hind*III, and *Pst*I (Pharmacia). The fragments were separated by electrophoresis in horizontal 0.5% agarose gels and stained with ethidium bromide. Electrophoresis was made in buffer C (90 mM Tris-HCl, 90 mM boric acid and 2.5 mM EDTA; pH 8.3) at 3.15 V/cm. As molecular size standard, lambda-DNA cut with Hind III/Eco RI (Pharmacia) was used.

Results

Culture and differentiation of hybrid plants

About 800 calli from different fusion products were obtained from experiments where the fusion products were isolated by micropipettes. From these, 13 calli (1.6%) differentiated into shoots and 5 of these were successfully transferred to the greenhouse. From the flow sorted experiments, 1,500 calli were obtained and 38 (2.5%) differentiated into shoots. Of these, 20 hybrid plants were obtained. From the total of 25 hybrid plants obtained, two were omitted from further analyses due to low viability.

Isozyme analysis

All of the shoots obtained from manually isolated fusion products showed hybrid character for the isozymes LAP and PGI (Sundberg and Glimelius 1986). Of the plants obtained from fusion products collected by flow sorting, 87% were hybrids while the rest were true *B. oleracea.*

Table 2. Hybrid morphology

Hybrid no.	Plant height at seed maturity, cm	Leaf morphology				Flower morphology
		Leaf shape	Hairiness	Wax	Thickness	
h1	95	Intermediate	+	–	+	Normal
h2	135	Intermediate	+ +	–	+	Big flowers
h3	115	Intermediate	+	–	+	Big flowers
h4	115	Intermediate	–	+	+ +	Normal
h5	120	Intermediate	–	+	+	Normal
h6	110	Intermediate	+	–	+	Normal
h7	30	Very small	–	+	+	Very small flowers
h8	110	Intermediate	–	+	+	Normal
h9	60	Intermediate	–	+	+ +	Normal
h10[a]	15	Very small	–	+	+ +	No flowers
h11	40	Intermediate	+	–	+	Small flowers
h12	95	Intermediate	–	+	+ + +	Big flowers
h13	110	Intermediate	+	–	+ + +	Big flowers
h14	90	Intermediate	+	–	+ + +	Big flowers
h15	30	Very small	–	–	+ + +	No petals, stigmas are small or missing
h16	60	Intermediate	+	+	+ +	Some flowers without stigmas
h17	115	*B. campestris* type	+	–	+	Flowers open before maturity
h18	105	*B. oleracea* type	–	+	+ + +	Big flowers
h19	100	*B. oleracea* type	–	+	+ + +	Big flowers
h20	95	*B. oleracea* type	–	+	+ + +	Big flowers, abnormal stamens
h21	30	Very small	–	+	+ +	Very small flowers
h22	30	Very small	+	–	+	Abnormal stigmas
h23	50	*B. campestris* type	+	–	+	Very big flowers
B. oleracea	50–80		–	+	+ +	
B. campestris	130		+	–	+	

[a] Has not reached maturity

Fig. 1. Different somatic hybrids at flowering. *First row* (*left to right*): h1, h4, h6 and h7 which all are hybrids with 38 chromosomes. *Second row* (*left to right*): four hybrids with 50–60 chromosomes (h10, h15, h16 and h20) and a chimera with approximately 38/76 chromosomes (h23)

Ploidy level of the hybrid plants

The chromosome numbers of the hybrid plants varied considerably (Table 1). Out of 23 analysed hybrids 7 (30%) had the expected amphidiploid chromosome number of 38 (20 chromosomes from *B. campestris* and 18 from *B. oleracea*). Two hybrids (9%) had slightly less than 38 chromosomes, 11 hybrids (48%) had 48–58 chromosomes and one hybrid (4%) contained 86 chromosomes.

According to the measurements of the relative DNA-content, two hybrids (9%) were chimeras (Table 1). In hybrid h22, leaf cells with relative DNA-values of 2.4 and 3.7 were found in equal amounts. The major part of the cells from leaves of hybrid h23 had a relative DNA-content of 4.8 while some cells had 2.4. When chromosome number in root tip cells were examined in these hybrids, only one cell type was found.

Analysis of pollen viability

Of the 7 hybrids with 38 chromosomes 6 had a pollen viability between 38% and 70% (mean 56%) (Table 1). One of the 38-chromosome hybrids had a pollen viability as low as 5%. The two hybrids with less than 38 chromosomes had pollen viabilities around 25%. For the hybrids with 48–58 chromosomes the amount of viable pollen varied from zero to 40% (mean 14%). The chimeras and the hybrid with chromosome number above 80 had a very low number of viable pollen (0–12%). Pollen viability for the parental species and *B. napus* varied between 67% and 75%.

Analysis of seed set

Seed set was very low for the hybrids with a chromosome number deviating from the expected 38 (Table 1). For hybrids with 48–58 chromosomes it was possible to obtain some seeds when they were pollinated with *B. napus*, but none or very few seeds were obtained after self-pollination. Within the group of hybrids with 38 chromosomes, seed set was very variable with a range of 1%–40% of the fertility of *B. napus*. Seed set measured as number of seeds/placenta varied between 0.003 and 0.115 after self pollination, 0.013 and 0.390 after pollination with *B. napus* and 0.016 and 0.152 after pollination with *B. campestris*. The chimerical hybrids did not produce and seeds. Seed set after self-pollination was 0.287 for *B. napus* and 0.327 for *B. oleracea*. Self pollination using buds instead of mature flowers did not increase the degree of fertilization except for the hybrid h4, where bud pollination was necessary for seed production.

Morphology

Morphology of the plants were variable especially within the group of plants with abnormal chromosome

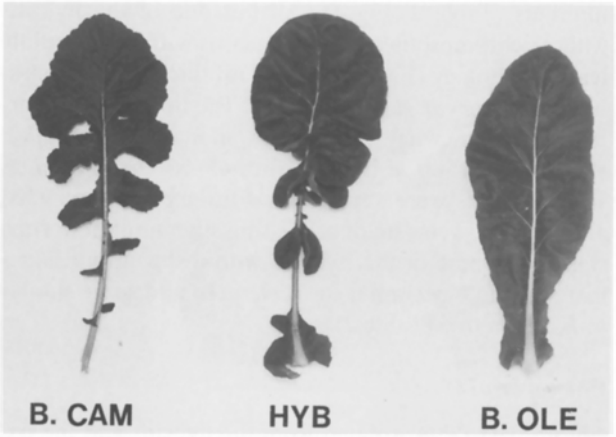

Fig. 2. Leaf morphology of *B. campestris*, *B. oleracea* "Blomkål" and an interspecific hybrid with 38 chromosomes

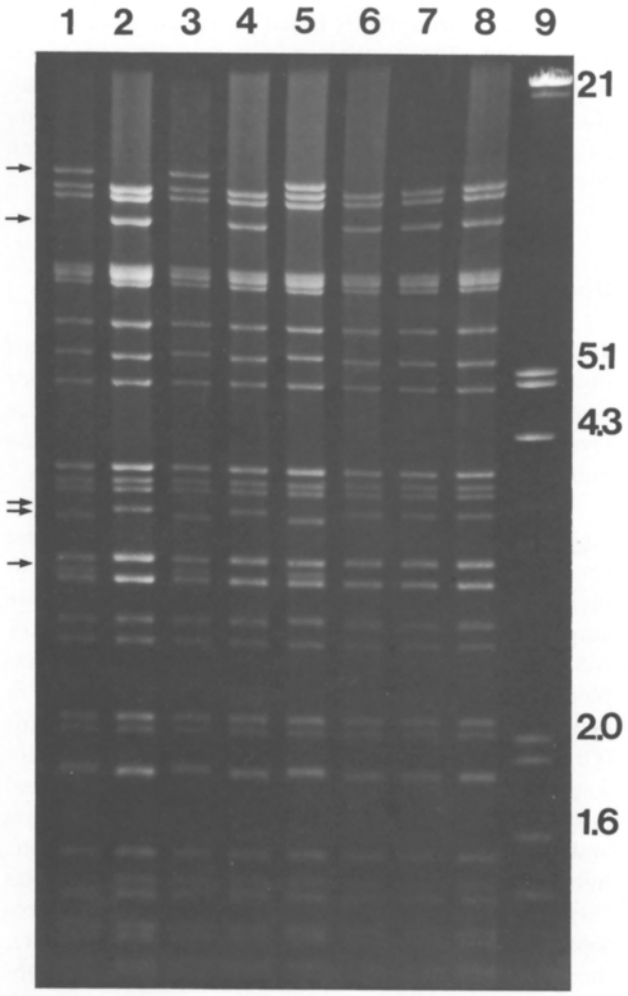

Fig. 3. Restriction pattern of chloroplast-DNA from parental and somatic hybrid plants with *Bam*HI restriction endonuclease. *Lane 1 B. campestris; lane 2 B.oleracea, lane 3* hybrid no 2; *lane 4* h6; *lane 5* h8; *lane 6* h12; *lane 7* h17; *lane 8* h23; *lane 9* molecular weight standard lambda-DNA cut with *Eco*RI/*Hind*III. The differences in restriction pattern between the parentals are indicated with *arrows*

numbers (Table 2, Fig. 1). All but one of the hybrids with 38 chromosomes were tall plants with intermediate leaf morphology (Fig. 2) and a plant thickness comparable to ordinary *B. napus* varieties. The flowers were normal, but somewhat larger than in natural *B. napus*. Within the group of hybrids with 48–58 chromosomes, several plants were very stout with large or somewhat abnormal flowers (small or missing stigmas or abnormal stamens). Some of the hybrids within this group had a leaf morphology similar to *B. oleracea* and some similar to *B. campestris* (Table 2).

Chloroplast DNA

Chloroplast-DNA isolated from the hybrids and the parental plants, listed in Table 1, was analysed with four different restriction endonucleases. Of 11 hybrids examined, 6 contained *B. oleracea* and 5 *B. campestris* chloroplasts. This grouping is based on results from all enzymes used. The restriction pattern of the parental species and some of the hybrids are illustrated with *Bam*HI (Fig. 3). There were no differences in restriction patterns between the various *B. oleracea* or between *B. campestris* varieties.

Discussion

The present investigation demonstrates that protoplast fusion of *B. oleracea* with *B. campestris* can be used for production of hybrids useful in plant breeding, since several of the hybrids had the expected chromosome number and were able to set seed.

A considerable variation in chromosome number among the obtained hybrid plants was observed. These results coincide with the work of Terada et al. (1987). In their investigation, 30% (3 out of 10 plants) were true amphidiploids, 50% had 56–57 chromosomes and 20% were chimeras. After sexual hybridization, when tetraploid *B. campestris* were crossed with tetraploid *B. oleracea,* most of the hybrids were true amphidiploids (Olsson 1960; Akbar 1987a, b). The increased chromosome variability in somatic hybrids compared to sexual is most probably due to the procedures used for fusion and regeneration of the fusion products. Somatic hybrids with 56 chromosomes could be fusion products between one *B. campestris* and two *B. oleracea* protoplasts as proposed by Terada et al. (1987). However, most of the deviation in chromosome number was probably induced during the cell culture stage. Newell et al. (1984) found that 40% of the plants derived from protoplasts of *B. napus* were diploids, 20% hypodiploids and 36% tetraploids or hypotetraploids.

Chromosomal balance and fertility of the plant is a prerequisite for good seed set. Since these traits are closely connected to pollen viability and this easily can be evaluated, an accurate method for analysis of pollen viability is of great value. In contrast to other methods used to estimate pollen viability (e.g. methylen blue, acetic carmine and anilin blue) the FDA-method utilized in this study measures the permeability of the plasma membrane, which is likely to be more closely related to viability than measurements of enzyme activity or stainability (Heslop-Harrison and Heslop-Harrison 1970; Rotman and Papermaster 1966). Estimates of pollen viability by the FDA-method are lower (70%, unpublished results) compared to e.g. methylen blue. For comparisons with other studies the figures given below are given in percentage of the corresponding estimates in *B. napus*.

Most of the hybrids produced by protoplast fusion in the current study showed an inferior pollen viability compared to a cultivated variety of *B. napus* and the parental species. Of the six amphidiploid somatic hybrids with normal morphology, 75% of the pollen was viable compared to natural *B. napus*. This agrees very well with the pollen viabilities in sexually synthesised hybrids between *B. oleracea* and *B. campestris* where the mean was 78% and 68%, respectively (Olsson 1960; Akbar 1987b).

In contrast, seed set was lower in this study compared to sexually synthesised rape. As expected, fertility of the hybrids with chromosome numbers deviating from the sum of the two parents was very low, as was that of the two chimerical hybrids found by DNA-content measurements. The hybrids with 38 chromosomes had 17% of the fertility of *B. napus* while the fertility of sexually synthesized *B. napus* was 53% compared to an ordinary *B. napus* variety (Akbar 1987a, b) which is close to what was found by Olsson (1960) and Inomata (1980). One reason for the lower fertility of the somatic hybrids compared to sexual hybrids could be chromosomal alterations induced by the in vitro culture. According to Newell et al. (1984) increased meiotic disturbances were found in plants derived from protoplasts of *B. napus* in spite of their normal chromosome numbers. In the present study this was also supported by the fact that the fertility of the somatic hybrids was improved when the hybrids were crossed with an already balanced *B. napus* variety. The differences found in fertility between the somatic and sexual hybrids might also depend on the different genotypes used in the hybridizations. Furthermore, the season when fertility tests were performed might also have influenced the seed set.

When producing rapeseed through sexual hybridization at the diploid level, *B. campestris* is almost always used as the female parent since the reciprocal cross is very hard to obtain although not impossible. Rapeseed resynthesised through sexual hybridization between diploid parents will thus normally have the

cytoplasm of *B. campestris*. However, when tetraploids of the parental species are used in sexual resynthesis of rapeseed, *B. oleracea* can also be used as the female parent without difficulties (McNaughton 1963). In the present study, all somatic hybrids examined contained either *B. oleracea* or *B. campestris* chloroplasts. The two kinds of hybrids were found in equal numbers. This is in good agreement with several other reports on the origin of chloroplasts in other somatic hybrids (Schiller et al. 1982; Iwai et al. 1980; Douglas et al. 1981; Bonnett and Glimelius 1983; Pelletier et al. 1983).

When comparing rapeseed resynthesised by somatic and sexual hybridization, a higher amount of variability was found in the somatic hybrids. This was especially evident for chromosome number, morphology and fertility. Again it can probably be explained by the effects of in vitro culture. Since the changes induced during culture of cells and calli in vitro are difficult to regulate, the only way to obtain genetically stable hybrids is to select them from a large population of hybrids. This is possible only if an efficient method for production and selection is available, which is the case using flow sorting for enrichment and accurate methods such as isozyme analysis, pollen viability tests and relative DNA-content measurements for screening the material.

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