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# Effects of parental ploidy level and genetic divergence on chromosome elimination and chloroplast segregation in somatic hybrids within *Brassicaceae*

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**Summary.** Chromosome and organelle segregation after the somatic hybridization of related species with different degrees of genetic divergence were studied by comparing the interspecific somatic hybrids *Brassica oleracea* (CC) (+) *B. campestris* (AA), *B. napus* (AACC) (+) *B. oleracea* (CC) *B. napus* (AACC) (+) *B. nigra* (BB) and *B. napus* (AACC) (+) *B. juncea* (AABB) with the intergeneric somatic hybrids *B. napus* (AACC) (+) *Raphanus sativus* (RR) and *B. napus* (AACC) (+) *Eruca sativa* (EE). Within each combination, some hybrids were found whose DNA content was equal to the sum of parental chromosomes, others had a relatively higher DNA content and in most of the cases, some had a relatively lower content. However, the frequency distribution in these three classes differed significantly between the combinations. A positive correlation between the frequency of hybrids with eliminated chromosomes and the genetic distance between the species in each combination was found. Furthermore, by combining species with different ploidy levels we found a significantly higher degree of chromosome elimination compared to combinations of species with the same ploidy level. In the *B. napus* (+) *B. nigra*, *B. napus* (+) *R. sativus* and *B. napus* (+) *E. sativa* combinations chromosomes from the B, R and E genomes appeared to be preferentially sorted out, as indicated by the fact that some of the nuclear markers from these genomes were missing in 7–46% of the plants, whereas no plants were lacking *B. napus* nuclear markers. Fertile hybrids were found in all but the *B. napus* (+) *R. sativus* fusion combination; the latter hybrids were male sterile, but female fertile. Hybrids between the A and C genomes were more fertile than hybrids obtained between the distantly related AC and B, R or E genomes, respectively. Analysis of the chloroplast RFLP pattern revealed that chloroplasts in the *B. oleracea* (+) *B. campestris* hybrids segregated randomly. A slightly biased segregation, favouring *B. napus* chloroplasts, was found in the *B. napus* (+) *B. oleracea* combination, whereas *B. napus* chloroplasts were strongly selected for in the *B. napus* (+) *B. juncea*, *B. napus* (+) *B. nigra*, *B. napus* (+) *R. sativus* and *B. napus* (+) *E. sativa* somatic hybrids.

**Key words:** Somatic hybrids – Brassicaceae – Genetic distance – Chromosome elimination – Chloroplast segregation

## Introduction

During the last decade somatic hybridization techniques have been developed for many different species (Gleba and Shlumukov 1990) and have proved to be a powerful tool for introducing genes from sexually incompatible species into a domesticated crop. Members of the family Brassicaceae, including the oil-yielding *Brassica* crops, can successfully be used in cell- and tissue culture (Vamling and Glimelius 1990), and several attempts to produce somatic hybrids between more or less distantly related species have been made (Gleba and Hoffmann 1980; Schenck and Röbbelen 1982; Sundberg et al. 1987; Toriyama et al. 1987a, b; Chatterjee et al. 1988; Fahleson et al. 1988a; Klimaszewska and Keller 1988; Primard et al. 1988; Sjödin and Glimelius 1989a; Kameya et al. 1989; Sikdar et al. 1990). Somatic hybrids were obtained in all of these cited studies, although ploidy level, chromosome elimination characteristics and fertility varies greatly between plants within each combination as well as between the different combinations.

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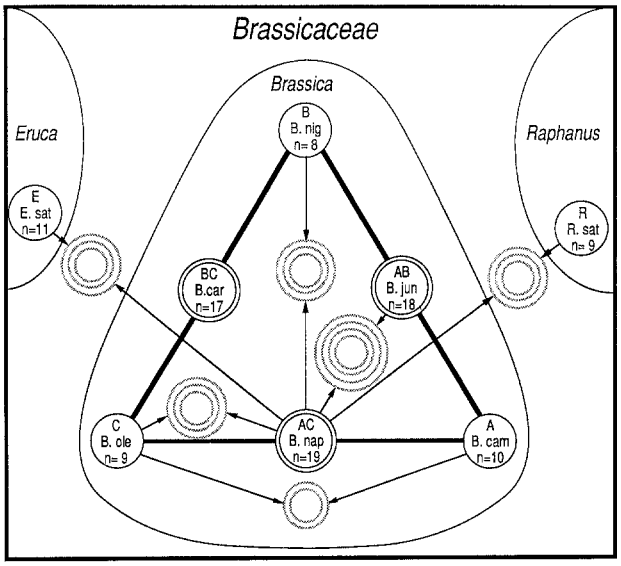


**Fig. 1.** A schematic drawing of the intra- and intergeneric somatic hybrids compared in this study. The somatic hybrids are respresented by *indented circles*. The number of circles represents the number of genomes in the species and hybrids. The *Brassica* species are arranged according to the triangle of U (U 1935) with *solid black lines* showing the phylogenetic relation between the allotetraploid and the diploid species

Biparental inheritance of organelles can be achieved via protoplast fusion. Unique combinations of cytoplasmic traits may consequently arise as a result of novel combinations of chloroplasts and mitochondria or through the rearrangement of organellar DNA. The chloroplast genotype in most regenerated somatic hybrids has been found to be one or the other of the two parental types (reviewed by Maliga and Menczel 1986). The mode of segregation of parental chloroplast types varies, depending on the species being combined. In some combinations the segregation of chloroplasts seems to be random (Chen et al. 1977; Fluhr et al. 1983), whereas in others it is biased, favouring one of the parental types (Glimelius et al. 1981; Bonnett and Glimelius 1983).

To study the influence of genetic divergence on chromosome elimination, fertility and chloroplast segregation, six populations of somatic hybrids between more or less distantly related species within the Brassicaceae were compared. All hybrids were produced with uniform and reproducible methods, allowing comparisons between the different combinations of species. The interspecific somatic hybrids *Brassica oleracea* (+) *B. campestris*, *B. napus* (+) *B. oleracea*, *B. napus* (+) *B. nigra* and *B. napus* (+) *B. juncea* were compared with the intergeneric somatic hybrids *B. napus* (+) *Eruca sativa* and *B. napus* (+) *Raphanus sativus* (Fig. 1). The fusion combinations analysed fall into the categories diploid (+) diploid, diploid (+) tetraploid and tetraploid (+) tetraploid, since *B. napus* and *B. juncea* are allotetraploids, whereas all the other species are diploids (Fig. 1). Thus, the present investigation also allowed a comparative analysis of the effect of ploidy level on chromosome elimination and chloroplast segregation.

## Materials and methods

### *Plant material*

The investigation was performed on six different combinations of somatic hybrids. Seeds of *B. napus* L. ssp. *oleifera* (2n = 38) cvs 'Hanna' and 'Korall', *B. oleracea* L. ssp. *botrytis* (2n = 18) cv 'Green Mountain', *B. juncea* L. (2n = 36) cv 'Varuna' and *Raphanus sativus* L. (2n = 18) cv 'Briljant' (with ogura cytoplasm) were used as parental material in the three fusions *B. napus* (+) *B. oleracea*, *B. napus* (+) *B. juncea* and *B. napus* (+) *R. sativus*. All seeds were kindly provided by W. Weibull AB, Svalöf AB and Hammenhög AB, Sweden.

The parental plant material used for the somatic hybrids *Brassica oleracea* (+) *B. campestris*, *B. napus* (+) *B. nigra* and *B. napus* (+) *Eruca sativa*, described by Sundberg et al. (1987), Sjödin and Glimelius (1989a) and Fahleson et al. (1988a, b), respectively, was *B. napus* L. ssp. *oleifera* (2n = 38) cv 'Hanna', *Brassica oleracea* L. ssp. *botrytis* (2n = 18) cv 'Green Mountain', *B. nigra* L. Koch (2n = 16) cv 'Junius' and *E. sativa* Mill. (2n = 22).

### *Protoplast isolation, fusion, selection and culture of hybrid cells*

Protoplasts were isolated from chlorophyll-containing, 5-day-old hypocotyls of *B. napus* and from etiolated, 5-day-old hypocotyls of either *B. juncea* or *R. sativus* for the fusions *B. napus* (+) *B. juncea* and *B. napus* (+) *R. sativus*. For the fusion *B. napus* (+) *B. oleracea* as well as for the already reported fusion combinations, protoplasts were isolated from etiolated hypocotyls and leaves of 3-week-old plants cultured in vitro. For details, see references for each combination. Plant material was cultured and protoplasts isolated according to Glimelius (1984) and Sjödin and Glimelius (1989a). Prior to fusion the etiolated hypocotyl protoplasts were stained with 5(6)-carboxyfluorescein for 10 min according to Sjödin and Glimelius (1989b). Protoplasts were fused using polyethylene glycol as described by Sundberg and Glimelius (1986). After fusion and washing, a solution was added containing W5 mixed 1 : 1 (v/v) with a modified 8p culture medium (Glimelius 1984) containing with 0.4 *M* mannitol and the cultures were stored at +8 °C in darkness for at least 2 h. Before selection of the heterokaryons by flow cytometry and cell sorting (Glimelius et al. 1986), the protoplasts were detached from the bottom of the petri dish, resuspended in W5 and centrifuged at 75 *g* for 5 min. The protoplast pellet was suspended in 8p medium containing 0.4 *M* mannitol and diluted to a density of $5 \times 10^5$ protoplasts/ml. After flow sorting, hybrid cells were cultured and plantlets regenerated according to Sundberg et al. (1991).

### *Confirmation of hybrid character by isoenzyme and RFLP analyses*

The nuclear composition of the regenerated plants was determined by isoenzyme and restriction fragment length polymorphism (RFLP) analyses. Isoenzyme analyses was performed according to Sundberg and Glimelius (1986) and the enzymes examined were leucine aminopeptidase (LAP), phosphoglucose isomerase (PGI), 6-phosphogluconate dehydrogenase (6-P) and phosphoglucomutase (PGM). As probes for RFLP analysis, we used a 570-bp *Hin*dIII-*Xho*I fragment from a napin gene (Ericsson 1988) and a 1800-bp *Pvu*II fragment of a cruciferin gene (Rödin et al. 1990). Total DNA was isolated from the hybrids according to a modified (Landgren and Glimelius 1990) protocol of

Bernatzky and Tanksley (1986). The Southern blot analysis was made according to a modified (Sjödin and Glimelius 1989b) protocol of Sambrook et al. (1989).

*Analysis of nuclear DNA content*

The nuclear DNA content of the hybrids was determined in a flow cytometer as described by Fahleson et al. (1988b). Leaf protoplasts were isolated from each hybrid plant 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 with flow cytometry as described by Fahleson et al. (1988b). The DNA content was determined as pg DNA/nucleus (Fahleson et al. 1988b).

*Analysis of chloroplast genotype*

For chloroplast DNA analysis, total DNA was isolated from the hybrids as described above. Total DNA was digested with *Bam*HI and resolved by electrophoresis. Chloroplast DNA restriction fragments could be distinguished from the nuclear smear on total DNA gels stained with ethidium bromide. Purified samples of chloroplast DNA of the parental species, isolated according to Sundberg et al. (1987), were used as standards.

*Seed set*

Seed set for each hybrid was recorded after self-pollination and backcrossing to the parental species. For each hybrid, 100 flowers were self-pollinated, and 50 flowers were fertilized with pollen from *B. napus* (cv 'Hanna' or 'Korall'). Fertility was expressed as the number of seeds obtained per pollinated flower.

*Data analysis*

A $\chi^2$ contingency analysis (Dixon and Massey 1969) was applied to test for independence between fusion combinations and DNA content.

To evaluate the effects of genetic distance and difference in ploidy level between the species in each combination on chromosome elimination, an analysis of covariance (Dixon and Massey 1969) was performed with difference in ploidy level as one factor and genetic distance as the regressor. The dependent variable (% elimination, Table 2) was arc-sine transformed (Dixon and Massey 1969). The fusions combining species with different ploidy levels (allotetraploid *B. napus* combined with the diploid *B. oleracea, B. nigra, R. sativus* and *E. sativa,* respectively) were considered as one group, and the fusions *B. oleracea* (+) *B. campestris* and *B. napus* (+) *B. juncea,* combining species with the same ploidy level, constituted the other group. Genetic distances based on RFLP data were obtained from Song et al. (1988, 1990). The genetic distances (arbitrary units) between A and C, AB and C and AC and AB genomes were taken from Song et al. (1988). To estimate the genetic distance between AC and B, R and E, respectively, averages of the A and C genomes genetic distances to each of the B, R and E genomes (Song et al. 1990) were calculated. Since the number of nuclear probes used by Song et al. in the 1988 study differed from the number used in 1990 (13 and 33 probes, respectively), the number of fragments differing between two species, and thus the genetic distance in the two studies, were not directly comparable. To utilize information from both studies the genetic distances from Song et al. (1988) were transformed by multiplying them by the ratio of the genetic distances between the A and C genomes in the two studies.

**Table 1.** Proportion of calli that regenerated shoots and plants with a hybrid pattern for at least one nuclear marker

| Somatic hybrid | No. of calli | Frequency (%) of calli regenerated shoots | No. of plants obtained | Frequency (%) of hybrid plants |
|---|---|---|---|---|
| *B.nap.* (+) *B.ole.* AACC CC | 1,128 | 8.9 | 67 | 93 |
| *B.ole.* (+) *B.cam.*[a] CC AA | 1,500 | 2.2 | 26 | 87 |
| *B.nap.* (+) *B.jun.* AACC AABB | 193 | 6 | 10 | 100 |
| *B.nap.* (+) *B.nig.*[b] AACC BB | 420 | 8.3 | 28 | 92 |
| *B.nap.* (+) *R. sat.* AACC RR | 364 | 5 | 11 | 100 |
| *B.nap.* (+) *E.sat.*[c] AACC EE | 1,052 | 6.2 | 54 | 63 |

[a] According to Sundberg et al. (1987)
[b] According to Sjödin and Glimelius (1989a)
[c] According to Fahleson et al. (1988a)

## Results

The porportion of calli that regenerated shoots varied from 2% to 9% between the different fusion combinations (Table 1). Isoenzyme and RFLP analyses revealed that 63%–100% of the plants obtained were hybrids (Table 1).

*Ploidy level*

Considerable variation in DNA content was found among the hybrids within each fusion combination as well as between the different combinations (Table 2). Hybrids were considered to have a DNA content equal to the sum of the parental chromosomes if they differed from the latter with no more than 6.9% (corresponding to 95% confidence interval assuming a standard deviation of 3.5%). Within each combination, the DNA content of some individuals was equal to the sum of the parental chromosomes; others had relatively higher DNA content and, in most cases, there were others with relatively lower content. The frequency distribution of hybrids in these three classes differed significantly ($\chi^2=71.6$; $df=10$; $P<0.001$) between the different fusion combinations. The covariance analysis showed that genetic distance and ploidy level differences between the parental species both had significant effects on chromosome elimination ($P=0.01$ and $P=0.02$, respectively). The model explains 95% of the variance in frequency of chromosome elimination ($r^2=0.94$). Regression lines for the two ploidy groups are shown in Fig. 2. In 7–46% of

**Table 2.** Ploidy level of the hybrid material measured by DNA-content analysis

| Hybrid combination | No. of hybrids | Sum of parental genomes | | Frequency (%) of hybrids with a DNA content corresponding to | | |
|---|---|---|---|---|---|---|
| | | Chromosome no. | DNA content (pg) | sum[d] | <sum | >sum |
| *B. nap.* (+) *B. ole.* AACC CC | 58 | 56 | 3.3 | 30 | 8 | 62 |
| *B. ole.* (+) *B. cam.*[a] CC AA | 23 | 38 | 2.15 | 33 | 10 | 57 |
| *B. nap.* (+) *B. jun.* AACC AABB | 8 | 74 | 4.15 | 88 | 0 | 12 |
| *B. nap.* (+) *B. nig.*[b] AACC BB | 30 | 54 | 3.3 | 69 | 24 | 7 |
| *B. nap.* (+) *R. sat.* AACC RR | 9 | 56 | 4.0 | 33 | 44 | 22 |
| *B. nap.* (+) *E. sat.*[c] AACC EE | 24 | 60 | 3.4 | 8 | 62 | 30 |

[a] According to Sundberg et al. (1987)
[b] According to Sjödin and Glimelius (1989a)
[c] According to Fahleson et al. (1988a)
[d] A hybrid was considered to have a DNA content equal to that of the sum of the parental chromosomes if it differed from the latter by no more than 6.9%

**Table 3.** Presence of nuclear markes in the different hybrids produced

| Hybrid combination | | No. of hybrids | No. of nuclear markers used[d] | Frequency (%) of hybrids showing | | |
|---|---|---|---|---|---|---|
| Parent | | | | Hybrid pattern for all markers | Parent-A pattern for at least one marker | Parent-B pattern for at least one marker |
| A | B | | | | | |
| *B. nap.* (+) *B. ole.* AACC | CC | 58 | 3 | 79 | 19 | 2 |
| *B. ole.* (+) *B. cam.*[a] CC | AA | 23 | 2 | 100 | 0 | 0 |
| *B. nap.* (+) *B. jun.* AACC | AABB | 8 | 2 | 88 | 0 | 12 |
| *B. nap.* (+) *B. nig.*[b] AACC | BB | 30 | 2 | 93 | 7 | 0 |
| *B. nap.* (+) *R. sat.* AACC | RR | 11 | 3 | 55 | 45 | 0 |
| *B. nap.* (+) *E. sat.*[c] AACC | EE | 24 | 3 | 54 | 46 | 0 |

[a] According to Sundberg et al. (1987)
[b] According to Sjödin and Glimelius (1989a)
[c] According to Fahleson et al. (1988a)
[d] The nuclear markers are two or three of the isoenzymes LAP, PGI, 6-P and PGM, or RFLP after Southern blot hybridization with the nuclear probes napin or cruciferin

the hybrids from the fusions between *B. napus* and the diploid species *B. oleracea*, *B. nigra*, *R. sativus* and *E. sativa*, respectively, some nuclear markers were missing from the C, B, R and E genomes (Table 3). Further, no or very few hybrids (2% of the *B. napus* (+) *B. oleracea* hybrids) had lost markers from the AC (*B. napus*) genome, indicating that chromosomes belonging to the diploid genomes were preferentially eliminated.

*Fertility of the somatic hybrids*

Meiosis of the hybrids was studied using fertility analysis. Self-fertile hybrids were found in all combinations except

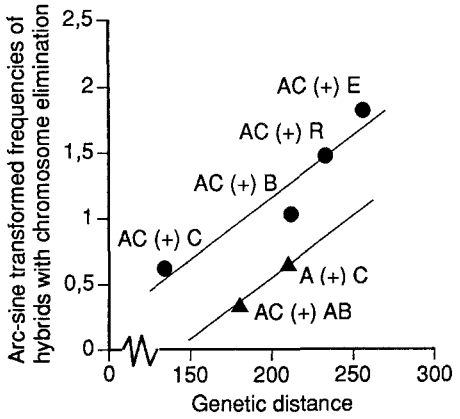


**Fig. 2.** The frequency (arc-sine-transformed) of hybrids having a lower chromosome number than the sum of parental chromosomes owing to chromosome elimination related to genetic distance of the species combined. Hybrids between species with different ploidy level are marked with *dots*, and hybrids between species with the same ploidy level are marked with *triangles*. The regression lines (estimated from covariance analysis) for the two different ploidy groups are included in the figure. See text for further details. *AC* (*B. napus*), *AB* (*B. juncea*), *A* (*B. campestris*), *C* (*B. oleracea*), *B* (*B. nigra*), *R* (*R. sativus*), *E* (*E. sativa*)

**Table 4.** Pattern of chloroplast segregation in the different combinations

| Hybrid type and chloroplast type (in brackets)[a] | | No. of hybrids | Frequency (%) of hybrids with chloroplasts from | |
|---|---|---|---|---|
| Parent A | Parent B | | Parent A | Parent B |
| *B. nap.* (+) (cam) | *B. ole.* (ole) | 58 | 72 | 38 |
| *B. ole.* (+) (ole) | *B. cam.* (cam) | 11 | 55 | 45 |
| *B. nap.* (+) (ole) | *B. jun.* (cam) | 6 | 100 | 0 |
| *B. nap.* (+) (cam) | *B. nig.* (nig) | 24 | 92 | 8 |
| *B. nap.* (+) (cam) | *R. sat.* (R. sat) | 11 | 91 | 9 |
| *B. nap.* (+) (cam) | *E. sat.* (E. sat.) | 17 | 88 | 12 |

[a] The allotetraploid *B. napus* and *B. juncea* can have either of the two chloroplast types of the parental species. *B. napus* cv 'Hanna' and cv 'Korall' have chloroplasts of *B. campestris* and *B. oleracea* type, respectively. *B. juncea* cv 'Varuna' has *B. campestris* type chloroplasts

the hybrids of *B. napus* (+) *R. sativus*, which were female fertile and male sterile. The genetic divergence between the species used for somatic hybridization also affects the fertility of the hybrids ($r=-0.9$; $P<0.01$). A fertility of 0.6–2.6 seeds per pollinated flower was recorded for the hybrids *B. napus* (+) *B. oleracea*, *B. oleracea* (+) *B. campestris* and *B. napus* (+) *B. juncea*. Much lower fertilities (0.1–0.4 seeds per pollinated flower) were found for the hybrids between *B. napus* and *B. nigra*, *R. sativus* or *E. sativa*, respectively. The fertility of hybrids with a chromosome number higher than the sum of parental chromosomes in the *B. napus* (+) *B. oleracea*, *B. oleracea* (+) *B. campestris* and *B. napus* (+) *B. nigra* combinations was significantly lower, indicating that the ploidy level of the hybrids also affects fertility (data not shown). Since backcrossing to *B. napus* has successfully been performed in all of the combinations studied, all combinations can be used for rapeseed crop improvement. Three of the combinations have already been integrated in rapeseed breeding programmes.

### *Chloroplast segregation*

The chloroplast DNA analysis revealed that chloroplasts randomly segregated in the *B. oleracea* (+) *B. campestris* combination. Segregation was slightly biased in favour of *B. napus* chloroplasts in the *B. napus* (+) *B. oleracea* combination, whereas *B. napus* chloroplasts were strongly selected for in *B. napus* (+) *B. juncea*, *B. napus* (+) *B. nigra*, *B. napus* (+) *R. sativus* and *B. napus* (+) *E. sativa* somatic hybrids (Table 4). The pattern of chloroplast segregation differed significantly ($\chi^2=11.4$, $df=5$, $P=0.04$) between the fusion combinations.

## Discussion

### *Ploidy level*

The results obtained in the present investigation indicate that both the degree of genetic divergence and differences in ploidy level between the species combined affect the frequency of chromosome elimination. Furthermore, the results indicate that the chromosomes of the diploid genomes were preferentially eliminated in the hybrids between the allotetraploid *B. napus* and the diploid *B. oleracea*, *B. nigra*, *R. sativus* and *E. sativa*.

One possible cause of chromosome elimination in hybrids could be that dissimilar nuclei differ in their cell-cycle times. For example, it has been suggested that if synthesis of the DNA of one of the genomes in a hybrid nucleus is still incomplete at the time for initiation of mitosis, chromatids might not have the ability to separate at anaphase, leading to the elimination of chromosomes (Gupta 1969). Differences in the time of completion of prophase condensation can also lead to chromosome elimination (Stutz 1962). Differences in cell-cycle time could increase with increasing genetic divergence. By comparing closely related cereals differing in ploidy level (e.g. hexaploid triticale compared with its tetraploid

and diploid parent species), Kaltsikes (1971) found that cell-cycle time decreased rather than increased as the nuclear DNA value increased. If the allotetraploid *B. napus* has a shorter cell-cycle time than the diploid species *B. oleracea*, *B. nigra*, *R. sativus* and *E. sativa,* respectively, the recovery of asymmetric hybrids that almost only miss nuclear markers from the C, B, R and E genome, respectively, could be due to the preferential sorting out of chromosomes from the parent with the longer cell-cycle time. Alternatively, since the culture and regeneration systems were developed for *B. napus* protoplasts, the tissue culture system used might select for hybrids with a complete *B. napus* nuclear genome.

Chromosome elimination could, besides being a result of genetic differences between the parental species – as suggested by the correlation between genetic distance and frequency of hybrids with eliminated chromosomes – also arise from the use of different cell types in the fusion experiments. Differences in cell-cycle time or cell-cycle phase, in which the cells are arrested when not dividing exist between different tissues. The somatic hybrids in the present study have primarily been produced by fusing protoplasts from hypocotyls containing both dividing and mature cells and protoplasts from leaf mesophyll with mostly arrested cells. Thus, the elimination of chromosomes might be a result of cell-cycle asynchrony between the different cell types fused. In a study (Sundberg et al. 1991) where somatic hybrids between *B. napus* and *B. oleracea* were produced, comparative fusions were performed, reciprocally, combining hypocotyl and mesophyll protoplasts as well as protoplasts of the same type. Frequencies of hybrid plants with a chromosome number less than that of the sum of the parental lines were similar for the two types of fusions (11.5% and 6% respectively, no significant differences). These results demonstrate that the elimination of chromosomes is due to species-specific differences rather than to cell-type specific differences.

*Chloroplast segregation*

The genetic divergence between chloroplast types of species within Brassicaceae (Palmer et al. 1983) is generally in accordance with the genetic divergence of their nuclear genomes (Song et al. 1988, 1990). In the present investigation the somatic hybrids produced between distantly related species tend to favour the chloroplasts contributed by the species predominating in the hybrid nucleus. Segregation generally occurred more randomly in the hybrid populations obtained from fusions of the more closely related species with more complete nuclear genomes. Thus, genetic divergence between the species combined in the present investigation might, through incompatibility reactions between nuclei and chloroplasts, influence the segregation of chloroplasts. In hybrids from the two closely related species *B. napus* and *B. juncea,* however, only *B. napus* chloroplasts were found. Still, only six plants were analysed, so the biased segregation of chloroplasts in this combination might, in fact, reflect haphazard events. In accordance with other investigations of somatic hybridization in which a deliberate selection pressure not was used for combining sequences of chloroplast DNA from the two chloroplast types, no hybrids with rearranged chloroplast DNA were found.

The biased segregation of chloroplasts could, besides being an effect of the degree of genetic divergence, also reflect differences in plastid DNA/total DNA ratios between the species used in the fusion experiments. Nuclear DNA content and cell size are two of the factors involved in determining plastid number and chloroplast DNA content (Butterfass 1989). An increase in the amount of nuclear DNA results in an increase in the number of plastids. Thus, the biased segregation favouring the *B. napus* chloroplasts found in four of the combinations in this study might have been due to an unequal input of organelles resulting from the fusion between the allotetraploid *B. napus* and the diploid *B. oleracea*, *B. nigra*, *R. sativus* or *E. sativa*. When the diploid species *B. oleracea* and *B. campestris* were fused, the chloroplasts segregated randomly. However, the biased segregation found in the hybrids between the two allotetraploid species *B. napus* and *B. juncea* cannot be ascribed to ploidy-level-related differences in plastid number since both species have approximately the same content of nuclear DNA. Differences in plastid number and DNA content per plastid have also been found between different tissues (Possingham et al. 1989). In the present investigation, different cell types, hypocotyl and leaf mesophyll were combined in the different fusion products. However, according to Sundberg et al. (1991) the cell type used in the fusion experiments has no significant effect on chloroplast segregation pattern. By contrast, results from studies of mitochondrial segregation suggest that mitochondria from the hypocotyl parent are favoured in the hybrids produced (Landgren and Glimelius 1990).

In conclusion, our findings show that the potential for utilizing somatic hybridization to improve the gene pool of *Brassica* crops is affected by the degree of genetic divergence between the species combined. As expected, chromosome elimination was more of a problem in hybrids between distantly related species than in hybrids between closely related ones. However, even in the combinations in which the elimination of chromosomes occurred frequently, most of the asymmetric hybrids obtained were fertile, enabling the transfer of genes from the hybrid to the crop.

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