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Inheritance of *Arabidopsis* DNA in offspring from *Brassica napus* and *A. thaliana* somatic hybrids

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Abstract Chromosome counts and RFLP markers mapped to *Arabidopsis thaliana* were used to determine the proportion of eliminated chromosomes and retained *A. thaliana* DNA in the back-crossed (BC) progeny derived from symmetric and asymmetric somatic hybrids between *Brassica napus* and *A. thaliana*. All plants were analysed for the presence of two RFLP markers per chromosome, preferably with one located on each chromosome arm. A reduction in both *A. thaliana* RFLP markers and chromosome numbers was found in the BC_1 and BC_2 generations of the symmetric hybrids as well as in the BC_1 generation of the asymmetric hybrids. In the symmetric hybrids, two back-crosses to *B. napus* were required to reduce the frequency of retained *A. thaliana* loci to 42.4% and mean chromosome number to 39.4. In comparison, the BC_1 progeny of the asymmetric hybrids had 16% of the analysed *A. thaliana* loci present and an average of 38.4 chromosomes maintained. When the frequency of *A. thaliana* chromosomes with both analysed loci maintained was compared with the frequency of chromosomes with one locus lost and one kept, a reduction in the number of complete chromosomes between BC_1 and BC_2 derived from the symmetric hybrids was observed. Among the BC_1 plants in the asymmetric group the situation was different, with higher amounts of incomplete donor chromosomes compared to whole chromosomes. The results indicate that *A. thaliana* chromosome fragments are more often found in the progeny of irradiated hybrids, while back-crossed symmetric hybrids have more complete chromosomes.

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Introduction

Mutation breeding as a supplement to conventional recombination breeding procedures has been under consideration since Muller (1925, 1927) and Stadler (1928) presented evidence that heritable changes in animals and plants could be induced. Many mutagenic agents efficiently induce chromosome breaks, which results in structural rearrangements of the chromosomal material. Some of these chromosomal rearrangements are transmitted through cell divisions, meiosis and carried through to the following generations. Sears (1956) showed that X-irradiation could be utilised to induce chromosomal rearrangements after sexual hybridisation to obtain a transfer of leaf-rust resistance from *Aegilops umbellulata* to bread wheat. Mutagenic treatments have been utilised in several cases where the aim has been to transfer genes to the genome of crop species from the chromosomes of distantly related species (Elliot 1957; Driscoll and Jensen 1964; Wienhues 1966; Sharma and Knott 1966; Knott 1968). Hence, it was logical to apply and investigate whether this method could be used for the transfer of limited amounts of donor DNA to a recipient plant genome after combination via somatic hybridisation (Dudits et al. 1980).

Morphological and cytological analyses have been the classical approaches to characterising sexual and somatic hybrids and their progeny. Cytological investigations have been improved by in situ hybridisation in which various probes are used to detect additional chromosomes and chromosomal integrations in the recipient genomes (Schwarzacher et al. 1989; Piastuch and Bates 1990). Furthermore, isoelectric focusing

(Hamill et al. 1985), isozymes (Gleba and Sytnik 1984; Sundberg and Glimelius 1986), species-specific DNA probes (Saul and Potrykus 1984), various molecular markers (Imamura et al. 1987; Gleba et al. 1988, Fish et al. 1988; Melzer and O'Connell 1990; Baird et al. 1992) and base pair heterogeneity (Neelam and Narayan 1994) are other tools used to investigate the genetic status of hybrid plants. However, detailed characterisations of somatic hybrids and their progeny regarding determination of the amount of retained donor DNA are rare. Only in genera where mapped restriction fragment length polymorphism (RFLP) markers are available, like in *Lycopersicon* (Wijbrandi et al. 1990; Melzer and O'Connell 1990, 1992), *Solanum* (Williams et al. 1990, 1993; Novy and Helgeson 1994; McGrath et al. 1996) and *Arabidopsis* (Forsberg et al. 1998a) have such analyses been performed.

In the study presented here, different strategies, such as back-crossing, irradiation or a combination of both, were used to analyse and compare the transfer and introgression of small amounts of donor DNA in progeny derived from symmetric and asymmetric somatic hybrids between *B. napus* and *A. thaliana*. The use of the well-characterised and densely mapped *A. thaliana* (Chang et al. 1988; Nam et al. 1989; Liu et al. 1996) as donor species for the hybrids provided us with a unique opportunity to follow which donor chromosomes were maintained in the genome and to evaluate the different procedures used to obtain fragmentation and introgression.

Materials and methods

Plant material

Production of the symmetric somatic hybrids between *B. napus* and *A. thaliana* is described by Forsberg et al. (1994). Ten back-crossed (BC) seeds per plant from six symmetric hybrids were sown. The six original hybrids had 48 chromosomes, the sum of the two parental species (*B. napus* 2n = 38, *A. thaliana* 2n = 10). These six symmetric hybrid plants were chosen for this study due to their chromosome number, access to seeds and DNA. From each primary symmetric hybrid, a number of BC_1 plants (totally 51) were investigated and further back-crossed (Fig. 1). Groups of BC_2 plants (totally 35), each deriving from 4 randomly selected individual BC_1 plants, were subsequently analysed. *B. napus* cv 'Hanna', was used as pollinator in all back-crosses in this study.

Asymmetric hybrids in which the donor species (*A. thaliana*) was UV-(Forsberg et al. 1998a) or X-irradiated (Forsberg et al. 1998b), were also included in this study (Fig. 1). Among the hybrids pre-treated with X- or UV-irradiation, 18 were considered as being asymmetric and 2 symmetric according to RFLP analyses. The asymmetric hybrids were further divided into hybrids with *A. thaliana* RFLP fragments of different sizes or hybrids with RFLP bands of the same size as those of the *A. thaliana* parent. In total 33 BC_1 offspring plants derived from 12 hybrids, where the donor protoplasts were X-irradiated with 1350 Gy, were included in the study. Two of these hybrids had a divergent RFLP pattern indicating potential rearrangements of the genomes. In addition 13 BC_1 plants obtained from 3 hybrids, where the donor protoplasts were irradiated with 800 Gy, were also included. The 3 primary hybrids obtained after pre-treatment with 800 Gy were chosen because they showed altered RFLP patterns compared to the parental material. Three BC_1 plants from 1 hybrid where the donor protoplasts were irradiated with 70 Gy and showed divergent RFLP pattern were also incorporated into the study. In addition, 12 BC_1 plants from 5 hybrids where the donor protoplasts were treated with UV irradiation (4680 J/m^2) were analysed. One of those exhibited a unique RFLP pattern with fragments of different size compared to the parents.

Chromosome analysis

Chromosome numbers were analysed in five to ten cells from four to eight root tips per plant according to Sundberg et al. (1987). In total 38 BC_1 plants and 30 BC_2 plants derived from the symmetric hybrids were analysed. Chromosome numbers were also determined in the BC_1 population of the asymmetric hybrids. Eleven progenies from the 4680-J/m^2 pre-treated group and 29 progenies from the 1350-Gy pre-treated group were included in the studies.

Plant DNA analysis

Isolation of plant DNA, Southern blot transfer and hybridisation were performed according to Sharpe et al. (1995) with modifications as described by Forsberg et al. (1998a). The DNA isolated from all plants was digested with *Eco*RI or *Eco*RV. The *A. thaliana* mapped RFLP markers mi100, mi353, mi444, mi277, mi289, mi456, mi30, mi123, mi322, mi418, mi372, mi259, mi320, mi403, mi122, mi334, mi121 and mi90 (Liu et al. 1996) were used as probes. In addition, the markers 104, 457 and 268 mapped by Chang et al. (1988) were included in the analyses. Different generations and subgroups were analysed over an extended period of time with respect to the inheritance of mapped *A. thaliana* loci. In the parental symmetric hybrids at least one marker per chromosome was analysed. The other plants were consistently analysed for the presence of two markers per chromosome, preferably with one located on each chromosome arm. The location of the markers on the *A. thaliana* chromosomes is partially illustrated by Forsberg et al. (1998b) including centromere positions (Round et al. 1997).

Statistical analyses

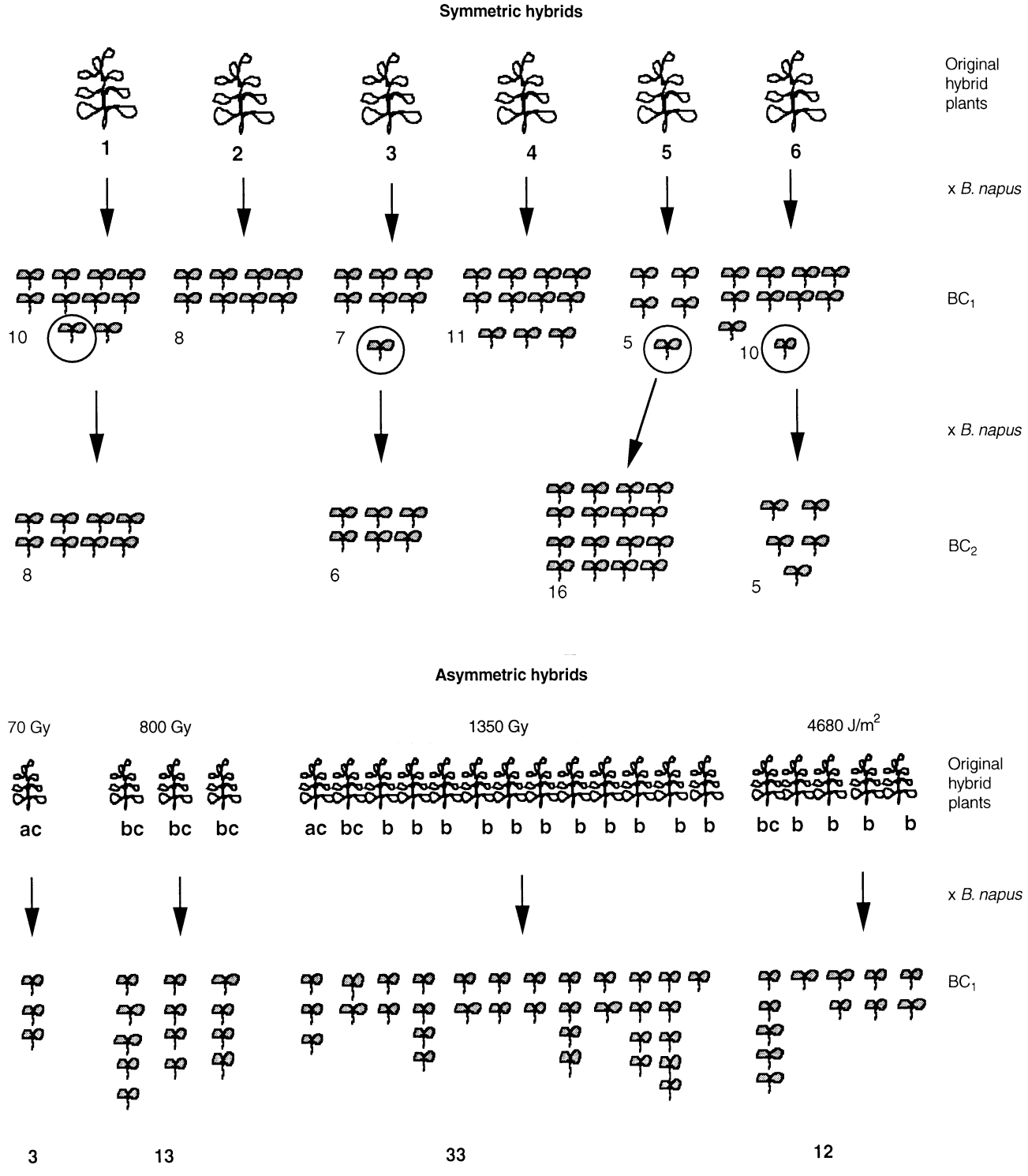
When the mean frequency of retained RFLP markers and number of chromosomes were determined, the mean value of each group of offsprings derived from an individual parent was used to calculate a mean for the whole generation. The differences in frequency of retained *A. thaliana* RFLP markers between generations of symmetric and asymmetric produced hybrids were analysed by Student's *t*-test.

Results

Characteristics of the analysed plant material

Five of the six original symmetric hybrids used in this study contained at least one RFLP marker for each

Fig. 1 A schematic drawing of the plant material used in the study. The asymmetric hybrids were X-irradiated with 70, 800 or 1350 Gy or UV-irradiated with 4680 J/m^2



a = all RFLP markers present
b = loss of RFLP markers
c = RFLP fragments of different size than *A. thaliana*

A. thaliana chromosome. The DNA obtained from the sixth hybrid plant was insufficient for further analysis.

Initially, 6 seeds from each of the hybrid plants, produced after pre-treatment of the donor, *A. thaliana*, with the highest irradiation doses of 1350 Gy and 4680 J/m^2, were placed on moist filter paper for germination. Seeds from 3 plants in each X- and UV-irradiated hybrid group failed to germinate even though up to 20 seeds per individual were tested. Additionally, offspring from 3 individuals in the 800-Gy pre-treated group and 1 individual in the 70-Gy pre-treated group, which all had aberrant RFLP patterns compared to *A. thaliana*, were chosen to be analysed. A clear reduction in germination rate was found in BC_1 both in the symmetric and asymmetric group of plants compared to the rapeseed cultivar 'Hanna'. The germination frequency was considerably increased after a second back-cross. The plants derived from the symmetric and the asymmetric hybrids, respectively, showed a highly variable morphology after one back-cross to *B. napus*. Individuals indistinguishable from the parental rapeseed cultivar and plants with more or less pronounced *A. thaliana* phenotypic characters (e.g. leaf shape, plant height, trichomes) were found. Seeds were obtained after both selfing and back-crossing and collected from all plants independent of background in the first and second back-crossed generations. Only 1 BC_1 plant, which derived from the 1350-Gy pre-treated group, did not develop flowers. A clear reduction in seed set compared to other BC_1 individuals was found in 3 plants deriving from the 1350-Gy group and in 2 plants deriving from the 800-Gy group.

RFLP analyses

The original asymmetric hybrids obtained after pre-treatment with 1350 Gy (Forsberg et al. 1998b) and 4680 J/m^2 (Forsberg et al. 1998a) did not differ significantly from each other with respect to presence of RFLP markers. Consequently, these two groups were treated as one asymmetric group in the statistical analyses in both the parental and in the BC_1 generation. The group obtained after pre-treatment with 70 Gy and 800 Gy were excluded from the comparisons since the hybrids chosen not were representative of their groups for number of *A. thaliana* loci maintained.

After the first back-cross the frequency of *A. thaliana* loci retained in the symmetric hybrids decreased to 94% (Fig. 2) with 17 plants out of 25 containing 100% of the *A. thaliana* loci analysed. Only the 2 BC_1 plants being parents to the BC_2 generation were analysed by RFLP markers. These 2 plants did not differ significantly from the rest of the BC_1 plants analysed in respect to RFLP markers, since both plants contained 100% of the analysed loci. In the offspring of the 2 BC_1 plants 78% of observed *A. thaliana* loci had been lost ($P < 0.01$, $t = 9.92$, $df = 3$) (Fig. 3a). By comparison,

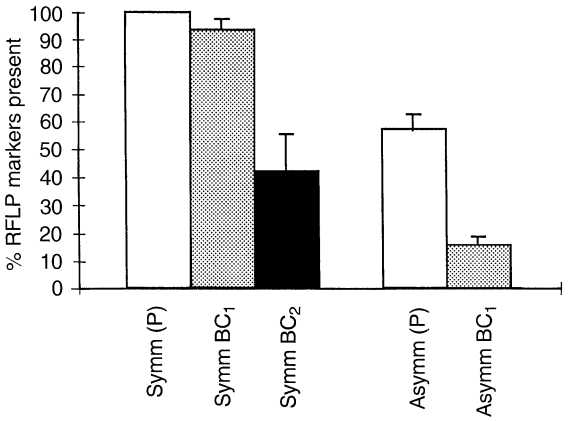


Fig. 2 Mean frequency of retained *Arabidopsis thaliana* RFLP markers in the somatic symmetric hybrids (*P*), the first and second back-crossed (*BC*) generation and the asymmetric hybrids (*P*) and their back-crossed offspring. The *white bars* represent original hybrids, and the *grey bars* the first generation and the *black bar* the second generation of hybrids. The standard error of the mean is added to all bars

the asymmetric group had already lost 73% ($P < 0.001$, $t = 3.65$ $df = 31$) of the markers analysed from the parental generation to BC_1 (Fig. 3b). Additionally, the frequency of retained *A. thaliana* loci after back-crossing of the symmetric hybrids was compared to the frequency in the asymmetric hybrids and their progeny. The BC_1 generation of the symmetric hybrids had 37% more loci maintained than the original asymmetric hybrids ($P < 0.01$, $t = 2.86$, $df = 20$). Furthermore, the symmetric BC_2 generation had 27% more markers compared to the asymmetric BC_1 generation ($P < 0.01$, $t = 2.88$, $df = 19$).

Since two *A. thaliana* loci per chromosome were analysed the difference between frequency of chromosomes maintained with both *A. thaliana* loci maintained, with one locus maintained and one lost or with both loci lost could be calculated. In the symmetric BC_1 plants, 91% of the chromosomes had both analysed *A. thaliana* loci maintained (Table 1). After a further back-cross the frequency had decreased to 37%. In the BC_1 plants of the asymmetric group, only 3.8% of the chromosomes had both analysed loci present and 22% had one locus maintained.

From 8 original asymmetric hybrids containing RFLP fragments which differed in size from *A. thaliana* (Fig. 4a), 34 BC_1 plants were analysed. In the analysed plants only one fragment with a unique size was inherited (Fig. 4b); all the other fragments had been lost.

Cytogenetic analyses

All six original symmetric hybrid plants contained 48 chromosomes. The first back-cross resulted in a

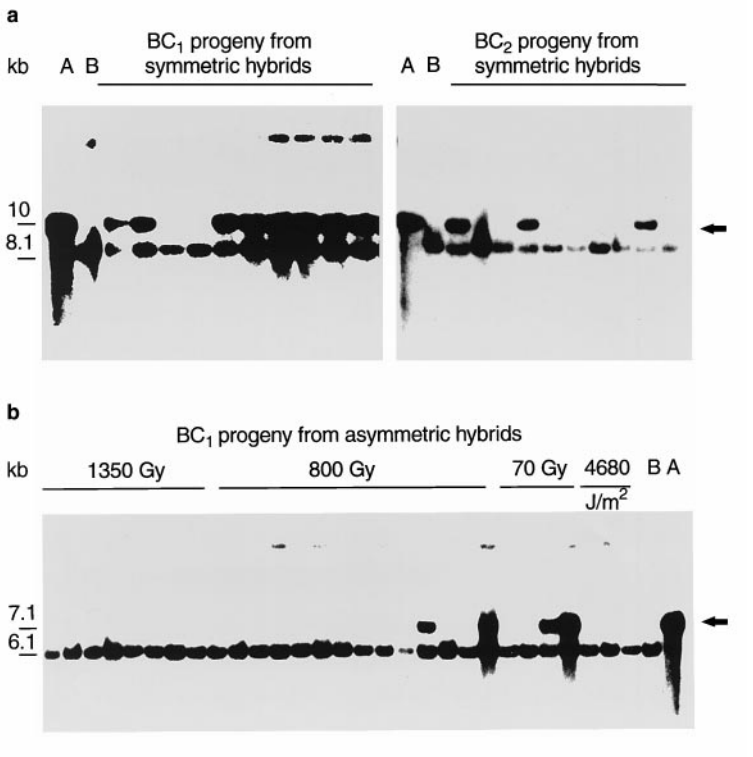


Fig. 3a, b Southern blot hybridisations of parental species *Arabidopsis thaliana* (*A*) and *Brassica napus* (*B*) and BC_1 and BC_2 progeny from symmetric hybrids (**a**) and BC_1 progeny from the asymmetric hybrids (**b**) using the mi418 marker as probe. Presence or absence of an *A. thaliana*-specific RFLP fragment (*arrows*) was recorded

Table 1 Chromosomes of plants in the different symmetric and asymmetric groups were analysed for the presence of two *A. thaliana* RFLP loci each. The data represent frequency of chromosomes with both *A. thaliana* loci maintained, one locus lost and one kept or both loci lost per chromosome

Material	Chromosomes with both loci maintained (%)	Chromosomes with one locus maintained and one lost (%)	Chromosomes with both loci lost (%)
Symmetric hybrids			
BC_1	91	6.4	2.4
BC_2	37	11	52
Asymmetric hybrids			
BC_1	3.8	22	74

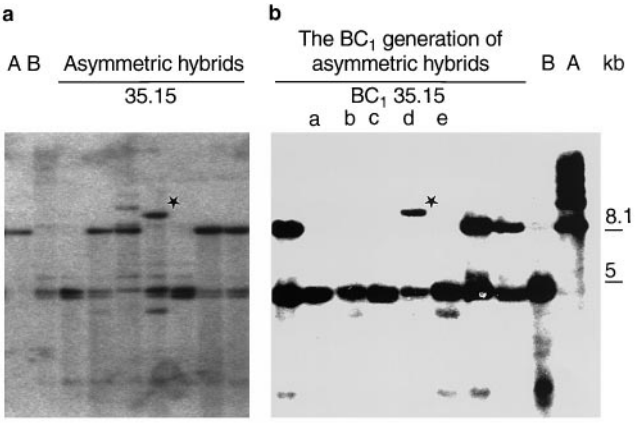


Fig. 4a, b Southern blot hybridisations of parental species *Arabidopsis thaliana* (*A*) and *Brassica napus* (*B*). **a** Parental asymmetric hybrids with one hybrid (35.15) showing an aberrant fragment (*). **b** BC_1 progenies from the asymmetric hybrids. Of five progenies from the 35.15 asymmetric hybrid (*a–e*), only one (35.15d) maintained the fragment of aberrant size (*). The marker mi30 was used as probe

significant decrease in chromosome number to a mean value of 42.8 ($P < 0.001$, $t = 4.8$, $df = 10$) (Fig. 5). None of the 4 BC_1 plants that were parents to the BC_2 generation were included in the chromosome analyses. However, the BC_2 group analysed had a decreased chromosome number ($x = 39.4$) compared to BC_1 ($P < 0.01$, $t = 3.7$, $df = 7$), indicating a rapid elimination of chromosomes. In the back-crossed asymmetric populations the mean chromosome number was 38.4, which was slightly lower than the value found ($x = 39.4$) in the symmetric BC_2 generation ($P < 0.01$, $t = 2.9$, $df = 19$).

Comparison between RFLP markers present and chromosome number

In the symmetric BC_1 and BC_2 generations and in the asymmetric BC_1 group, a number of plants ($n = 65$) were analysed both for chromosome number and presence of RFLP markers (Fig. 6). The BC_1 generation of

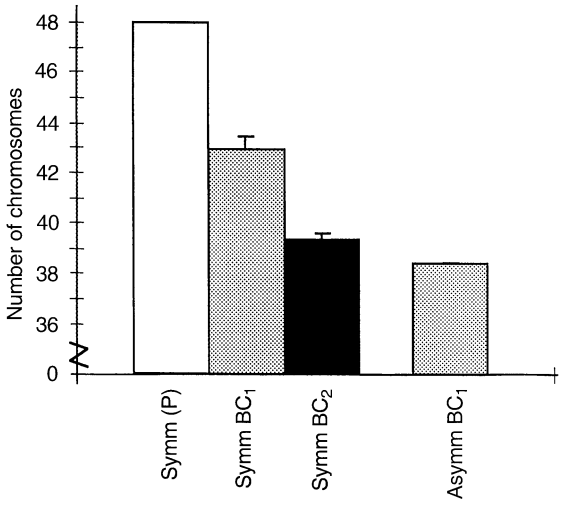


Fig. 5 Mean number of chromosomes in the symmetric hybrid (*P*), the two generations of back-crossed (*BC*) symmetric hybrids and in the first generation of back-crossed asymmetric hybrids. The *white bar* represents the original hybrid and the *grey bars* the first generation and the *black bar* the second generation of hybrids. The standard error of the mean is added to all bars

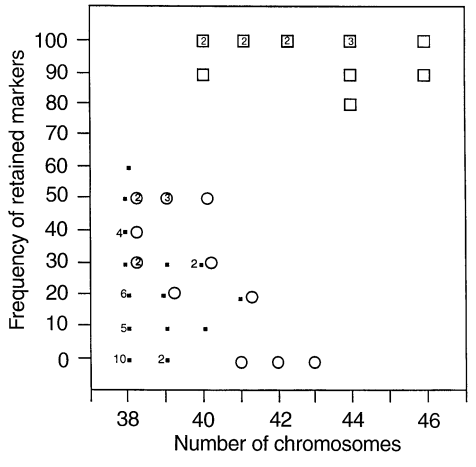


Fig. 6 The frequency of retained *A. thaliana* loci plotted against the number of chromosomes in the first (□) and second (○) generation of back-crossed symmetric hybrids and in the first (■) generation of back-crossed asymmetric hybrids. The *numerals in* or *beside* the symbols represent the number of plants when more than one plant from the same group has the same value

the symmetric hybrids showed a higher frequency of both retained *A. thaliana* loci and chromosome numbers than the BC_2 generation and the BC_1 of the asymmetric hybrids. Individuals were found in all three groups, which had more RFLP markers than the expected number of chromosomes. Additionally, in 3 BC_2 plants deriving from the symmetric hybrids a chromosome number higher than that of *B. napus* (38) was detected without any *A. thaliana* loci observed.

Discussion

To follow the integration and elimination of *A. thaliana* DNA in the genomic background of *B. napus*, we compared progeny from two back-crossed generations of symmetric somatic hybrids between *B. napus* and *A. thaliana* with progeny from one back-crossed generation derived from asymmetric somatic hybrids. After one back-cross of the symmetric hybrids a high frequency of *A. thaliana* loci was retained from the parental symmetric hybrid plants. This indicates that a large number of *A. thaliana* chromosomes were present in the BC_1 genomes. This conclusion was further confirmed by the cytological examinations of BC_1 where the mean chromosome number was found to be 42.8; i.e. on average the *B. napus* chromosome number plus 5 extra chromosomes. After the second back-cross, the number of *A. thaliana* loci were considerably reduced in the progeny derived from the symmetric hybrids. Several of the absent loci were mapped to the same chromosome, indicating that the elimination of complete chromosomes had taken place. This assumption was further strengthened by the substantial reduction in chromosome number. In the back-crossed populations of the asymmetric plants, RFLP analysis indicated the presence of fewer intact *A. thaliana* chromosomes and a higher number of chromosomes with only one of two analysed loci present compared with the symmetric group. This observation correlated well with the cytological examinations, which showed a clear reduction in chromosome number.

When results from the RFLP analysis of the back-crossed asymmetric plants were compared with the original asymmetric hybrid from the 1350-Gy (Forsberg et al. 1998b) and 4680 J/m^2 (Forsberg et al. 1998a) pre-treated groups, a reduction in the number of chromosomes with both *A. thaliana* loci maintained was found. This reduction is, per se, expected after a back-cross. The ratio between the frequency of chromosomes with some loci lost and some maintained in the asymmetric group compared to the frequency of chromosomes with all analysed loci maintained had increased after one back-cross from 2 to 6. This indicates that an increased number of recombinations between the two genomes had taken place. This can be compared with the situation found in the symmetric group where this ratio increased from 0.07 in BC_1 to 0.3 in BC_2. The relative increase in lost markers in the two groups is similar to asymmetric values and indicates that the loss of *A. thaliana* loci occurs in a similar way when asymmetric and symmetric BC_1 hybrids are back-crossed. However, even if the increase in the symmetric back-cross is comparable with the asymmetric values the relative numbers are considerably lower.

Only 1 plant out of 34 analysed in the BC_1 generation of the asymmetric group inherited an *A. thaliana* RFLP fragment that was different in size to that of the

A. thaliana parent. All other aberrant fragments were lost in the back-cross. This indicates that most of the aberrant fragments were not present due to stable rearrangements, e.g. translocations in the *B. napus* genome. It is worth noting that in 95% of the RFLP analyses performed in this study and in all our investigations of the original hybrids (Forsberg et al. 1998a,b), *Eco*RI was used, which has been shown in some cases to be methylation-sensitive (Jen-Jacobson et al. 1996). Consequently, possible methylation in the plant material interfering with the RFLP analyses can be responsible for the variable number of novel fragments observed in the original hybrids. The fact that one fragment was inherited does not exclude the possibility that it was also methylated in the next generation. In conclusion, more detailed investigations are needed to elucidate this further.

Chromosome loss is a common event in interspecific sexual and somatic hybridisation. In the asymmetric group, chromosome elimination and rearrangements were assumed to be induced by mutagenic treatments in combination with back-crosses. The loss of chromosomes in the back-crossed asymmetric group was compared with the loss in the BC_1 and BC_2 symmetric hybrids. A significant elimination of chromosomes was the result after the first back-crossed symmetric generation and a further decrease in chromosome number was found in BC_2. A mean chromosome number of 42.8 found in the BC_1 generation of the symmetric hybrids fits the theoretically calculated value obtained after one back-cross, whereas the BC_2 value of 39.4 is close to the expected value of BC_3, indicating a rather rapid reduction in the number of chromosomes. The preferable elimination of *A. thaliana* chromosomes was, furthermore, visualised by the phenotype of the two generations where the BC_2 plants showed a morphology more alike to *B. napus* than the BC_1 plants. By comparison, the mean chromosome numbers found in BC_1 plants of the asymmetric hybrids rather fit the theoretical values calculated for the BC_5 generation, demonstrating a relatively direct effect of the mutagenic pre-treatments on chromosome eliminations.

In the present study two RFLP markers per chromosome were analysed in the hybrids. Subsequently, the amount of *A. thaliana* loci retained in the hybrid plants should be correlated to the number of chromosomes, assuming that no DNA or chromosomal disturbances have occurred. This was also, in general, found in the plants analysed, independent of origin. However, some of the analysed plants had 38 chromosomes and, in addition, high levels of retained donor loci. This result indicates that intergenomic translocations or, alternatively, the substitution of *B. napus* chromosomes with *A. thaliana* chromosomes, might have occurred. Indeed, intergenomic translocations have previously been shown in asymmetric somatic hybrids between *B. napus* and *Lesquerella fendleri* where translocations of *L. fendleri* into the *B. napus* genome were found (Skarzhinskaya et al. 1998). Additionally, in our material we found plants with 41–43 chromosomes that had no analysed *A. thaliana* markers present. This result might be explained by the occurrence of DNA methylation or the loss of the *A. thaliana* DNA where the RFLP markers were mapped. However, the accuracy of the cytological examinations in this study is estimated to be plus/minus one chromosome due to their small size and the large number of chromosomes in each cell. Hence, the determination of whether certain chromosome arms were lost or if different sets of chromosomes were present in any of the plants studied was not possible without more detailed investigations, e.g. in situ hybridisation.

In conclusion, even if fertile symmetric somatic hybrids can be obtained between *B. napus* and *A. thaliana*, treatments causing a fragmentation of the donor genome (*A. thaliana*) seems to be advantageous in reducing the amount of donor DNA and promoting recombination between the two genomes in a short period of time.

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References

Baird E, Cooper-Bland S, Waugh R, DeMaine M, Powell W (1992) Molecular characterization of inter- and intra-specific somatic hybrids of potato using random amplified polymorphic DNA (RAPD) markers. Mol Gen Genet 233:469–475

Chang C, Bowman JL, DeJohn AW, Lander ES, Meyerowitz EM (1988) Restriction fragment length polymorphism linkage map for *Arabidopsis thaliana*. Proc Natl Acad Sci USA 85: 6856–6860

Driscoll CJ, Jensen NF (1964) Characterisation of leaf rust resistance transferred from rye to wheat. Crop Sci 4:372–374

Dudits D, Fejer O, Hadlaczky G, Koncz C, Lazar GB, Horvath G (1980) Intergeneric gene transfer mediated by protoplast fusion. Mol Gen Genet 179:283–288

Elliott FC (1957) X-ray induced translocation of *Agropyron* stem rust resistance to common wheat. J Hered 38:77–81

Fish N, Karp A, Jones MGK (1988) Production of somatic hybrids by electrofusion in *Solanum*. Theor Appl Genet 76:260–266

Forsberg J, Landgren M, Glimelius K (1994) Fertile somatic hybrids between *Brassica napus* and *Arabidopsis thaliana*. Plant Sci 95:213–223

Forsberg J, Dixelius C, Lagercrantz U, Glimelius K (1998a) UV dose-dependent DNA elimination in asymmetric somatic hybrids between *Brassica napus* and *Arabidopsis thaliana*. Plant Sci 137:65–76

Forsberg J, Lagercrantz U, Glimelius K (1998b) Comparison of UV-light and restriction enzyme treatment as tools in production of asymmetric somatic hybrids between *Brassica napus* and *Arabidopsis thaliana*. Theor Appl Genet 96:1178–1185

Gleba YY, Sytnik KM (1984) Protoplast fusion. Genetic engineering in higher plants. Springer, Berlin Heidelberg New York

Gleba YY, Hinnisdaels S, Sidorov VA, Kaleda VA, Parokonny AS, Boryshuk NV, Cherep NN, Negrutiu I, Jacobs M (1988) Intergeneric asymmetric hybrids between *Nicotiana plumbaginifolia* and *Atropa belladonna* obtained by "gamma-fusion". Theor Appl Genet 76 : 760–766

Hamill JD, Pental D, Cocking EC (1985) Analysis of fertility in somatic hybrids of *Nicotiana rustica* and *N. tabacum* and progeny over two sexual generations. Theor Appl Genet 71 : 486–490

Imamura J, Saul MW, Potrykus I (1987) X-ray irradiation promoted asymmetric somatic hybridization and molecular analysis of the products. Theor Appl Genet 74 : 445–450

Jen-Jacobson L, Engler LE, Lesser DR, Kurpiewski MR, Yee C, McVerry B (1996) Structural adaptations in the interaction of *Eco*RI endonuclease with methylated GAATTC sites. EMBO J 15 : 2870–2882

Knott DR (1968) *Agropyron* as a source of rust resistance in wheat-breeding. Ed. by Finlay KW and Shephard KW, In: Proc 3rd Wheat Genetics Symp. Canberra: Aust Acad of Sci, pp 204–212

Liu Y-G, Mitsukawa N, Lister C, Dean C, Whitter RF (1996) Isolation and mapping of a new set of 129 RFLP markers in *Arabidopsis thaliana* using recombinant inbred lines. Plant J 10 : 733–736

McGrath JM, Wielgus SM, Helgeson JP (1996) Segregation and recombination of *Solanum brevidens* synteny groups in progeny of somatic hybrids with *S. tuberosum*: intragenomic equals or exceeds intergenomic recombination. Genetics 142 : 1335–1348

Melzer JM, O'Connell MA (1990) Molecular analysis of the extent of asymmetry in two asymmetric somatic hybrids of tomato. Theor Appl Genet 79 : 193–200

Melzer JM, O'Connell MA (1992) Effect of radiation dose on the production of and the extent of asymmetry in tomato asymmetric somatic hybrids. Theor Appl Genet 83 : 337–344

Muller HJ (1925) The regionally differential effect of X-rays on crossing over in autosomes of Drosophila. Genetics 10 : 470–507

Muller HJ (1927) Artificial transmutation of the gene. Science 66 : 84–87

Nam H-G, Giraudat J, den Boer B, Moonan F, Loos WDB, Hauge BM, Goodman HM (1989) Restriction fragment length polymorphism linkage map of *Arabidopsis thaliana*. Plant Cell 1 : 699–705

Neelam A, Narayan RKJ (1994) Studies of backcross progeny of *N. rustica* × *N. tabacum* interspecific hybrids II. Genomic DNA analysis. Cytologia 59 : 385–391

Novy RG, Helgeson JP (1994) Somatic hybrids between *Solanum etuberosum* and diploid, tuber bearing *Solanum* clones. Theor Appl Genet 89 : 775–782

Piastuch WC, Bates GW (1990) Chromosomal analysis of *Nicotiana* asymmetric hybrids by dot blotting and *in situ* hybridization. Mol Gen Genet 222 : 97–103

Round EK, Flowers SK, Richards EJ (1997) *Arabidopsis thaliana* centromere regions: genetic map positions and repetitive DNA structure. Genome Res 7 : 1045–1053

Saul MW, Potrykus I (1984) Species-specific repetitive DNA used to identify interspecific somatic hybrids. Plant Cell Rep 3 : 65–67

Schwarzacher T, Leitch AR, Bennett MD, Heslop-Harrison JS (1989) In situ localizaation of parental genomes in a wide hybrid. Ann Bot 64 : 315–324

Sears ER (1956) The transfer of leaf-rust resistance from *Aegilops umbellulata* to wheat. Brookhaven Symp Biol 9 : 1–21

Sharma D, Knott DR (1966) The transfer of leaf rust resistance from *Agropyron* to *Triticum* by irradiation. Can J Genet Cytol 8 : 137–143

Sharpe AG, Parkin IAP, Keith DJ, Lydiate D (1995) Frequent nonreciprocal translocations in the amphidiploid genome of oilseed rape (*Brassica napus*). Genome 38 : 1112–1121

Skarzhinskaya M, Fahleson J, Glimelius K, Mouras A (1998) Genome organization of *Brassica napus*, *Lesquerella fendleri* and analysis of their somatic hybrids using genomic *in situ* hybridisation. Genome 41 : 691–701

Stadler L (1928) Genetic effects of X-rays in maize. Proc Natl Acad Sci USA 14 : 69–75

Sundberg E, Glimelius K (1986) A method for production of interspecific hybrids within *Brassicaceae* via somatic hybridization, using resynthesis of *Brassica napus* as a model. Plant Sci 43 : 114–162

Sundberg E, Landgren M, Glimelius K (1987) Fertility and chromosome stability in *Brassica napus* resynthesised by protoplast fusion. Theor Appl Genet 75 : 96–104

Wienhues A (1966) Transfer of rust resistance of *Agropyron* to wheat by addition, substitution and translocation. Ed. by Mackey J, In: Proc 2nd Int Wheat Genetics Symp. Uppsala. Hereditas, pp 328–341

Wijbrandi J, Zaber P, Koornneef M (1990) Restriction fragment length polymorphism analysis of somatic hybrids between *Lycopersicon esculentum* and irradiated *L. peruvianum*: evidence for limited donor genome elimination and extensive chromosome rearrangements. Mol Gen Genet 222 : 270–277

Williams CE, Hunt GJ, Helgeson JP (1990) Fertile somatic hybrids of *Solanum* species: RFLP analyses of a hybrid and its sexual progeny from crosses with potato. Theor Appl Genet 80 : 545–551

Williams CE, Wielgus SM, Haberlach GT, Guenther C, Kim-Lee H, Helgeson JP (1993) RFLP analysis of chromosomal segregation in progeny from an interspecific hexaploid somatic hybrid between *Solanum brevidens* and *Solanum tuberosum*. Genetics 135 : 1167–1173