Theor Appl Genet (1998) 96: 1178–1185

J. Forsberg · U. Lagercrantz · K. Glimelius

Comparison of UV light, X-ray and restriction enzyme treatment as tools in production of asymmetric somatic hybrids between *Brassica napus* and *Arabidopsis thaliana*

Received: 18 December 1997 / Accepted: 7 January 1998

Abstract Asymmetric somatic hybrids between *Brassica napus* (receptor) and *Arabidopsis thaliana* (donor) have been produced by three different methods supposed to induce asymmetry. The donor protoplasts were either UV- or X-irradiated, or the mixture of protoplasts was treated with the restriction enzyme *Pvu*II immediately before fusion. The genome composition of the hybrids was analysed with Southern blot hybridisations using 15 different mapped *A. thaliana* RFLP markers as probes. Both UV- and X-irradiation were found to be efficient treatments for induction of asymmetry in somatic hybrids in a dose-dependent manner. The addition of a restriction enzyme to the protoplast mixture did not have any effect on the frequency of asymmetric hybrids or on the degree of asymmetry in the hybrids produced. UV- and X-irradiation resulted in higher fertility in the hybrids, while *Pvu*II treatment did not have any effect on seed set. A significant positive correlation between degree of asymmetry in different plants and seed set after selfing was detected.

Key words Asymmetric somatic hybridisation · Mapped RFLP markers · Restriction endonuclease · Ultraviolet irradiation · X-irradiation

Introduction

Somatic hybridisation has the potential to be used as a plant breeding technique due to its possibility to combine sexually incompatible species (Waara and Glimelius 1995). In most cases it is of interest to transfer only a limited amount of genetic material from a foreign species to a crop rather than to combine two complete genomes, i.e. it is desirable to create asymmetric rather than symmetric somatic hybrids. Different techniques for the induction of such asymmetry have been developed. Since the first report of Dudits et al. (1980) the most commonly utilised method has been to expose the isolated donor protoplasts to ionising irradiation, either γ-irradiation (Bates et al. 1987) or X-irradiation (Dudits et al. 1980), prior to fusion. This treatment is performed to increase fragmentation and integration of the donor chromosomes into the receptor genome and has resulted in intergenomic translocations (Piastuch and Bates 1990; Hinnisdaels et al. 1992; Parokonny et al. 1992; Skarzhinskaya et al. 1998). Recently, exposure of protoplasts to UV-irradiation has been reported as a suitable treatment to fragment and eliminate chromosomes (Jazdzewska et al. 1995; Vlahova et al. 1997). However, the methods need further development to achieve an extensive elimination of donor DNA combined with an integration of the remaining donor genome in the receptor genome. If this could be achieved a stable inheritance of the transferred traits could be the result.

Another attractive strategy to obtain stable highly asymmetric hybrids could be the use of restriction endonucleases to fragment the donor DNA. Restriction endonucleases are enzymes that cleave DNA at specific recognition sites thus creating DNA double-strand breaks. Such breaks have been shown to generate chromosomal aberrations, and the treatment and its results have been extensively studied in mammalian cells (Bryant 1984; Thacker 1994). There are also two reports on chromosome breaks after restriction enzyme treatment in plant cells (Subrahmanyam et al. 1976; Stoilov et al. 1996). In these examples with mammalian and plant cells, the chromosomal aberrations, were studied but the effects of the restriction enzymes on survival and regeneration of the cells were not followed.

Communicated by Yu. Gleba

J. Forsberg (✉) · U. Lagercrantz · K. Glimelius
Department of Plant Biology, Uppsala Genetic Center; Swedish University of Agricultural Sciences, Box 7003, S-750 07 Uppsala, Sweden

Nevertheless, restriction enzymes are particularly interesting as tools in asymmetric somatic hybridisation, since the primary effect of enzyme treatment would probably be chromosomal aberrations such as intergenomic translocations. In contrast, X- and UV-irradiation at high doses are known to be detrimental to the cell and result in a high frequency of point mutations. These effects could influence the frequency of surviving hybrid cells and the amount of mutations in the resulting asymmetric hybrids.

The species *B. napus* and *A. thaliana* have been used as model material for the studies. *A. thaliana* is useful as a model donor species in somatic hybridisation since it has a small genome with few chromosomes that are densely covered with restriction fragment length polymorhic (RFLP) markers (Chang et al. 1988; Nam et al. 1989; Liu et al. 1996). This provides unique opportunities to make an efficient analysis of the genomic constitution of the hybrids. In the present study, the different experiments for production of somatic hybrids between *B. napus* and *A. thaliana* have been performed identically, except for the restriction enzyme treatment, X- or UV-irradiation of the protoplasts. The study has been designed in such a way that the effects of treatments and doses applied to the donor protoplasts could be compared. The comparisons have been focused on the frequency of asymmetric hybrid plants, degree of asymmetry in the plants and influence of asymmetry on fertility.

Materials and methods

Protoplast fusion and regeneration

The production of hybrids with UV-irradiation is described elsewhere (Forsberg et al. 1997). The different irradiation doses used were 780 J/m^2 (156 J/m^2 · min, 5 min.), 2340 J/m^2 (15 min.) and 4680 J/m^2 (30 min.). The selection system was based on *A. thaliana* transformed with the *bar* gene (Thompson et al. 1987), which confers resistance to the herbicide Basta® (Hoechst). A concentration of 100 mg/l Basta in all culture media prevented the growth of unfused *B. napus* protoplasts. This was combined with a selection procedure preventing *A. thaliana* from growing since unfused protoplasts could not proliferate in the culture system developed for *B. napus*. Shoot regeneration frequencies were determined as the proportion of calli transferred to shoot inducing medium that differentiated into shoots.

The same fusion and selection methods were used when the *A. thaliana* protoplasts were X-irradiated. The protoplasts were floated in CPW 16 (Banks and Evans 1976) during the irradiation (Sjödin and Glimelius 1989). A Siemens Stabilipan 200 X-ray machine was used, and the tube (TR 200f) was operated at 180 kV and 10 mA. The irradiation was filtered through 1 or 4 mm Al, resulting in a dose rate of 7.5 or 4.5 Gy/min, respectively. Three different doses were applied: 70 Gy (1 mm Al, 7.5 Gy/min, 9.3 min.), 800 Gy (4 mm Al, 4.5 Gy/min, 178 min.) and 1350 Gy (1 mm Al, 7.5 Gy/min, 180 min.).

In the restriction enzyme-treated experiments the same methods for isolation of protoplasts, fusion and regeneration of plants were used . *Pvu*II in the concentrations 100, 250, 500, 1000 and 2000 U/ml of protoplast suspension (900 000 cells/ml) was added to the mixture of protoplasts directly before the PEG-(polyethylene glycol) induced fusion. Highly concentrated *Pvu*II (50 U/µl, Amersham) was used. Control experiments were also performed, with addition of an equivalent amount (10–200 µl) of restriction enzyme storage buffer [10 m*M* TRIS-HCl, 50 m*M* KCl, 0.1 m*M* EDTA, 10 m*M* 2-mercaptoethanol, 0.01% BSA and 50% glycerol (pH 7.5)] per milliliter protoplast suspension. One experiment in which the protoplasts were fused without any pre-treatment was also included in this study.

DNA analysis

Isolation of plant DNA, Southern blot transfer and hybridisation were done according to Sharpe et al. (1995) with details as described in Forsberg et al. (1997). Fifteen different mapped *A. thaliana* RFLP markers were used as probes (Fig. 1) (Liu et al. 1996). In addition, a 980-bp fragment from the *bar* gene construct was used as a probe. Hybrids missing at least one *A. thaliana* specific RFLP band were classified as asymmetric.

Fertility

The fertility of the hybrids was examined by selfing and back-crossing to *B. napus* (male parent). When possible, 100 flowers per hybrid were self-pollinated and 50 flowers per hybrid back-crossed. The number of seeds produced per pollinated flower was calculated.

Statistical analysis

The correlation between irradiation dose and the degree of asymmetry in the hybrids was tested with linear regression. The degree of asymmetry was expressed as the frequency of *A. thaliana* loci outsorted in the hybrids. To reduce the dependence between frequency and variance, the frequencies were arc-sine transformed (Dixon and Massey 1969). A Spearman rank correlation (Lehmann 1975) was performed between the frequency of markers scored

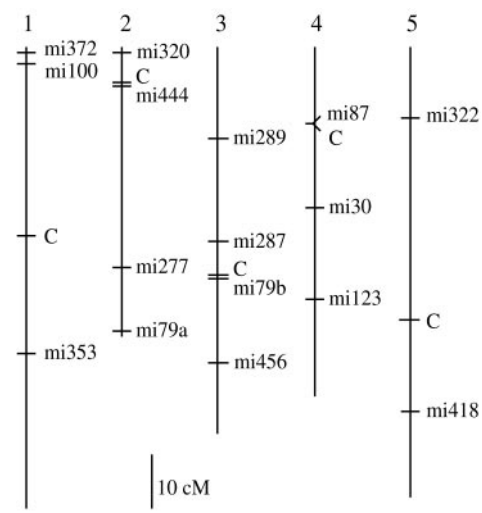

Fig. 1 A schematic linkage map indicating the positions of the *A. thaliana* RFLP markers used in the analyses (Liu et al. 1996). Loci mi79a and mi79b were both detected with marker mi79a. The centromere (C) positions of the different chromosomes are indicated in the figure (Schmidt 1995; Zachgo et al. 1996; Round et al. 1997)

absent from the total number of markers analysed and the seed set after self-fertilisation.

Results

Regeneration

Regeneration frequency varied between 1% and 13% among the different experiments (Table 1). A total of 838 shoots were produced and 298 hybrid plants were included in fertility analysis. DNA analyses were performed on 168 plants with 15 mapped markers (Fig. 1) resulting in an evaluation of a total of 2184 data points (Fig. 2). In control experiments the addition of restriction enzyme storage buffer alone did not have any negative effect on fusion and callus growth.

Frequency of asymmetric hybrid plants produced

Both UV- and X-irradiation proved to be powerful tools in the production of asymmetric somatic hybrids. The frequency of asymmetric hybrid plants produced varied between 57% and 100% in the experiments where UV- and X-irradiation were used as pre-treatments (Fig. 3). Irradiation from both sources resulted in higher frequencies of asymmetric hybrids compared to the control. In contrast, the *Pvu*II treatment had no statistically significant effect on the frequency of asymmetric hybrids produced. With the exception of the group treated with 2000 u/ml, which only contained 2 plants of which 1 was asymmetric, the frequency of asymmetric hybrids in the restriction enzyme-treated group varied between 23% and 25%. The frequency of asymmetric hybrids in the control group was 10%. The *bar* gene was present in all hybrids analysed.

Degree of asymmetry in the produced hybrids

When the degree of asymmetry in the hybrids was considered, the use of UV- or X-irradiation was found to be efficient in the production of asymmetric somatic hybrids, resulting in a limited transfer of donor DNA to the hybrids. The average frequency of analysed markers retained in each plant varied between 49% and 95% for the different irradiation doses (Fig. 4). The plants with the lowest amounts of retained *A. thaliana* DNA were found in the UV-irradiated material, while the plants from the X-irradiated experiments showed a more clear-cut dose response. Both the UV- and X-irradiated experiments showed a significant positive correlation between the irradiation dose and the degree of asymmetry (Forsberg et al. 1997 and Fig. 5). The degree of asymmetry in the hybrids produced after *Pvu*II treatment did not differ significantly from the control experiment. The mean frequency of analysed markers retained in each plant from the *Pvu*II treated experiments were 95% and 97%, while the control mean was 99.6% (Fig. 4).

Table 1 Treatments, number of calli transferred to shoot regeneration media, number of regenerated shoots, shoot regeneration frequencies and number of plants included in DNA and fertility analysis

Treatment	Calli transferred to shoot induction	Regenerated shoots		Number of plants included in analyses	
		Number	Frequency (%)	DNA	Fertility
Control[a]	1 523	115	8	20	51
Restriction enzyme (U/ml)					
100	1 488	101	7	26	43
250	1 072	86	8	20	29
500 (I)[b]	650	11	2	1	2
500 (II)[b]	55	2	4	0	0
1 000	440	11	2	1	3
2 000	429	27	6	2	4
UV-irradiation (J/m^2)[a]					
780	831	110	13	26	48
2 340 (I)[b]	215	26	12	4	8
2 340 (II)[b]	476	25	5	1	5
4 680	2 465	36	1	7	7
X-irradiation (Gy)					
70	970	116	12	14	44
800	2548	120	5	30	34
1 350	1 915	52	3	16	20
Total	15 077	838		168	298

[a] The results from the UV-irradiated experiments as well as the control experiment without pre-treatment have earlier been reported in Forsberg et al. (1997)
[b] (I) and (II) are different experiments with the same treatment

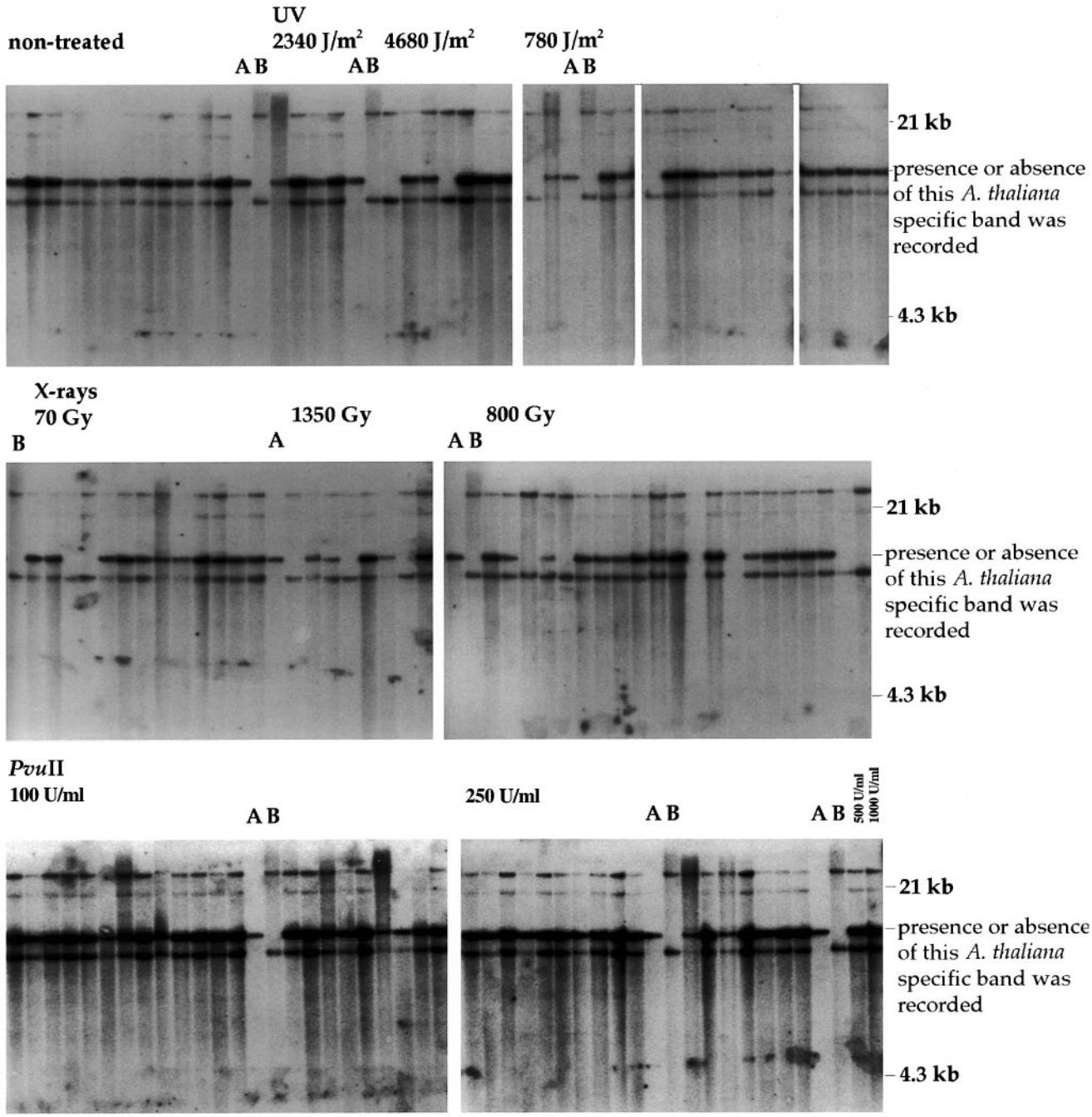

Fig. 2 Autoradiograms with hybridisation of the mi418 probe to *Eco*RI digested DNA from somatic hybrids, *A. thaliana* (A) and *B. napus* (B). The different treatments and doses are indicated in the figure

When the degree of asymmetry of the plants from the highest doses of each treatment (*Pvu*II: 250 U/ml; UV: 4680 J/m^2; X-rays: 1350 Gy) was compared, it was revealed that plants produced with UV- or X-irradiation did not differ significantly from each other in degree of asymmetry. The same was true for the *Pvu*II treated and control plants. Those two pairs of treatments, however, did differ clearly from each other with respect to the degree of asymmetry in the hybrids (Fig. 6).

Chromosome fragments and rearrangements

Marker data indicated that 30% of the chromosomes in the hybrids produced with pre-treatment were partial. At least 2 RFLP markers from each *A. thaliana* chromosome were analysed. If at least one but not all markers from a particular *A. thaliana* chromosome were present in the hybrid, it indicated that part of the chromosome was missing, and such chromosomes were

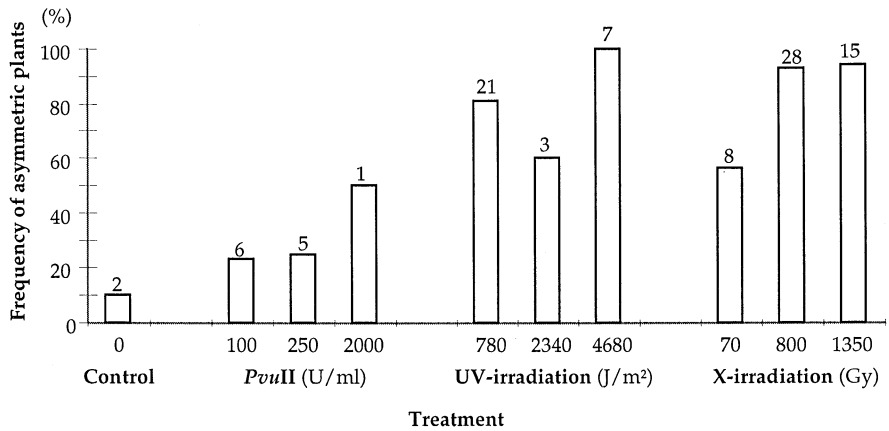

Fig. 3 Frequency of asymmetric hybrids produced without pre-treatment (0), with *Pvu*II treatment, UV- or X-irradiation. The numbers on the top of the bars indicate the number of plants represented by each bar

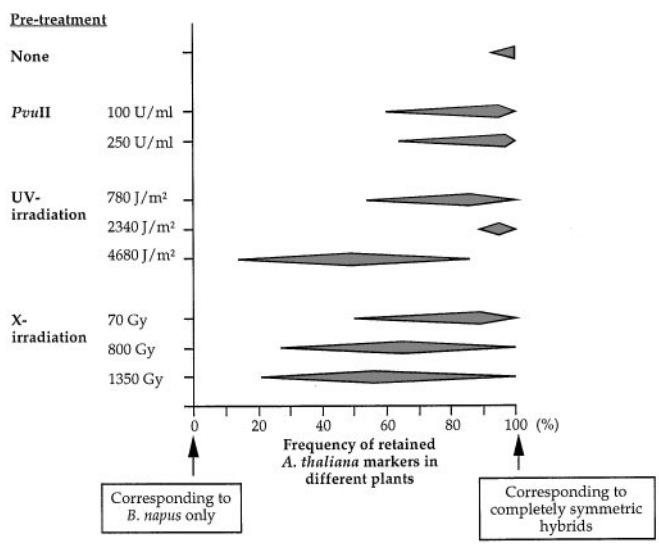

Fig. 4 Average, minimum and maximum frequencies of retained *A. thaliana* markers in different plants produced with the various treatments. The thickest parts of the areas represent the average. In this figure, data from ten plants have been excluded since fewer than 7 markers were analysed for each one

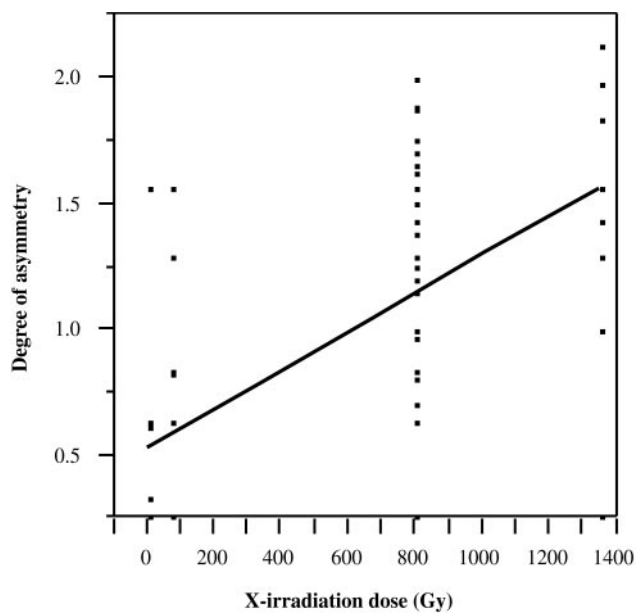

Fig. 5 Linear regression of the degree of asymmetry expressed as the arc-sine-transformed frequency of markers scored absent in the different hybrids, versus X-irradiation dose ($t = 8.32$, $df = 79$, $P < 0.001$ and $r^2 = 0.47$)

considered as partial in the analysis. Five percent of the analysed chromosomes lacked all scored markers, indicating that whole chromosomes were missing. In the two experiments with the highest irradiation doses of UV- and X-irradiation respectively, marker data indicated that 4680 J/m^2 resulted in 49% partial chromosomes and 23% absent (Forsberg et al. 1997) and that 1350 Gy resulted in 56% partial chromosomes and 15% absent. These data indicate that chromosome fragmentation occurs frequently and that the partial loss of *A. thaliana* chromosomes is more common than the complete loss of donor chromosomes. Eleven plants from the UV- and X-irradiated experiments, as well as 1 control plant, showed RFLP bands that differed in size from those in the *A. thaliana* parent. Both the mapped mi markers and the transformed *bar* fragment showed such alterations in fragment size. The frequency of such plants was similar in the X- and UV-irradiated experiments. In the restriction enzyme-treated experiment no plants with RFLP bands of aberrant sizes were found.

Fertility and morphology

No obvious morphological differences between plants from different treatments and doses were observed. However, when symmetric and asymmetric hybrids

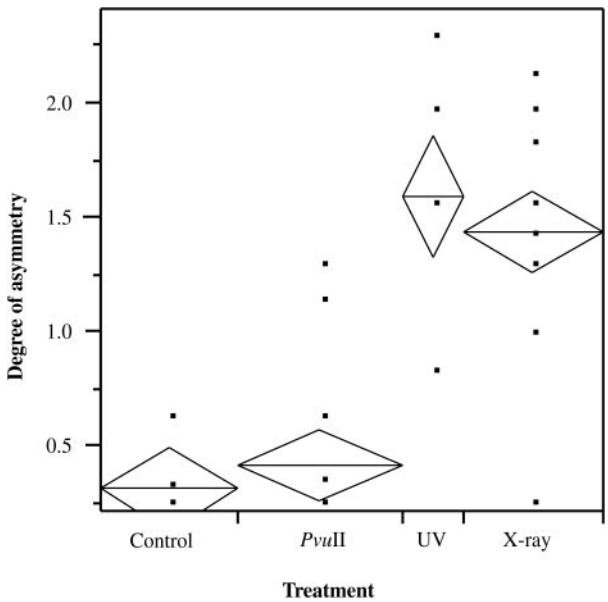

Fig. 6 The degree of asymmetry expressed as the arc-sine-transformed frequencies of outsorted markers in the different hybrids from the highest doses of each treatment (250 U/ml *Pvu*II, 4680 J/m^2 UV-irradiation and 1350 Gy X-irradiation). The width of the squares indicates the number of plants included in each group and the height of the squares shows the 95% confidence interval for each treatment

were compared, the asymmetric hybrids were slightly taller and had larger leaves. The fertility after back-crosses to *B. napus* was not affected by the different treatments and neither could female fertility be correlated to the degree of asymmetry in the hybrids. In contrast, the fertility, after selfing was higher in UV- or X-irradiated hybrids (Fig. 7). Higher irradiation doses resulted in higher fertility, and the Spearman rank correlation test between seed set after selfing and degree of asymmetry (using all analysed plants) showed a highly significant positive correlation ($\rho = 0.44$, $P < 0.001$) (Fig. 8).

Discussion

The aim of this study was to evaluate and compare different methods for the induction of chromosome fragmentation and elimination in order to produce asymmetric somatic hybrids. By studying chromosome elimination with mapped *A. thaliana* RFLP markers spread over the chromosomes, we were able to estimate the amount of donor DNA in the hybrid genome. UV- and X-irradiation proved to be efficient means of inducing asymmetry and producing high frequencies of asymmetric hybrid plants. In contrast, restriction enzyme treatment did not result in any significant elimination of DNA from the donor protoplasts.

We still believe that restriction enzymes could be an attractive means to produce asymmetric hybrid plants, and that the method should be modified and tested further. The single enzyme, *Pvu*II, used in the present

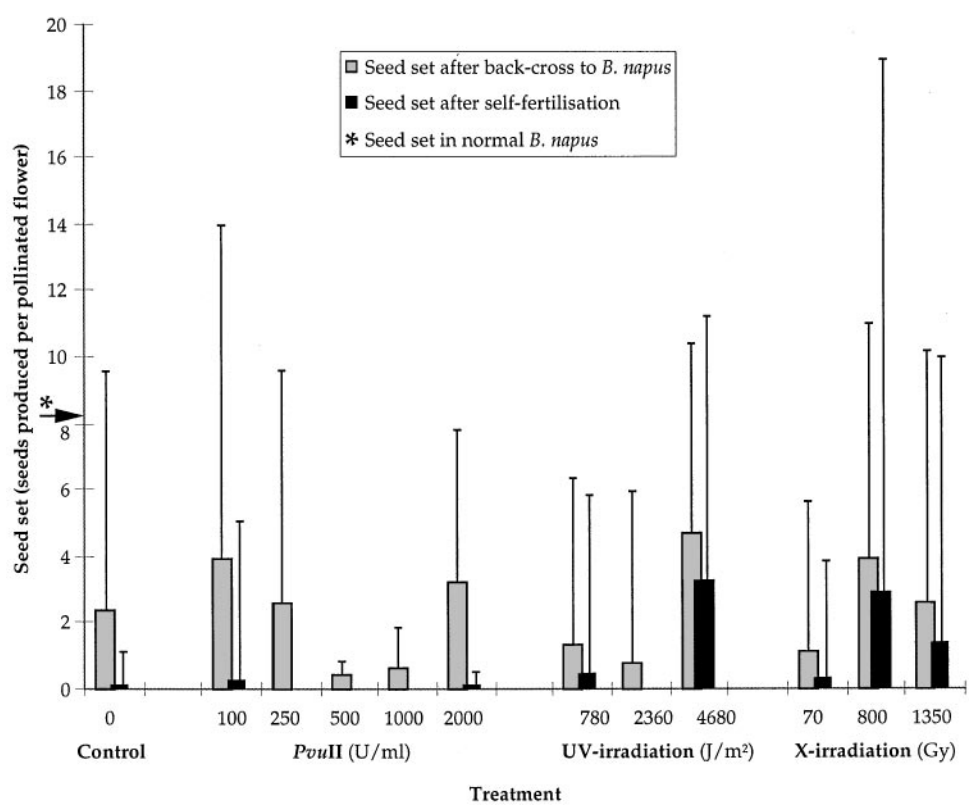

Fig. 7 Average seed set after back-crossing to *B. napus* and selfing of the hybrids produced with the different treatments. The lines indicate the maximum value produced within each treatment. Sterile hybrids were found in all groups

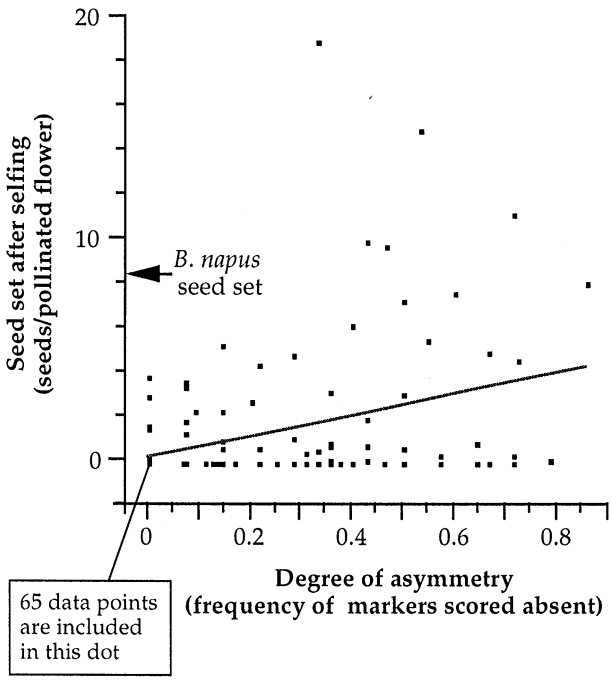

Fig. 8 Seed set after selfing plotted against degree of asymmetry represented as the frequency of markers scored absent for each plant. Plants from all different treatments are included. The estimated regression line is indicated

study was chosen because it has been reported to be effective in creating chromosomal aberrations in earlier studies (Bryant 1984; Natarajan and Obe 1984; Costa and Bryant 1990; Moses et al. 1990; Costa and Bryant 1991; Costa et al. 1993). In the present study, the conditions were optimised for the plant protoplasts rather than for the restriction enzyme to ensure a sufficient survival of the cells. For example, the salt concentration was too high for the enzyme. In future studies other enzymes that are more active in the protoplast media should be tested. Electroporation of the protoplasts in the presence of restriction enzyme to enhance uptake of the enzyme is also an option. This method has been reported to increase the effect of added restriction enzymes in mammalian cells (Winegar et al. 1989). The optimal conditions for DNA cleavage could also be evaluated by pulsed field gel electrophoresis prior to fusion.

X-irradiation at high doses is the most efficient way to produce a large number of asymmetric hybrids with a high degree of asymmetry. UV irradiation, at high doses, is also a very good alternative. Both irradiation types result in the elimination of partial rather than complete chromosomes, at similar frequencies, irrespective of irradiation type. However, when UV-irradiation is used, the low shoot regeneration frequency (Forsberg et al. 1997) might be a bottle-neck, and probably a larger number of experiments have to be performed to achieve the same number of asymmetric hybrids as with X-irradiation. The use of UV-irradiation, therefore, probably demands more resources than X-irradiation in order to achieve the same results.

The production of asymmetric somatic hybrids as opposed to symmetric hybrids has been suggested to be a way to produce more fertile hybrids (Bates et al. 1987), which has also been indicated in other studies (Hinnisdaels et al. 1994). The results obtained in this investigation and a previous one (Forsberg et al. 1994) support this assumption. We obtained a significant positive correlation between degree of asymmetry and fertility after selfing, which means that the total fertility increased in asymmetric hybrids.

Besides fragmentation and the elimination of donor chromosomes, introgression of donor DNA in the receptor genome is a goal in asymmetric somatic hybridisation. Intergenomic translocation facilitates the stable inheritance of the transferred traits. Among the UV- and X-irradiated plants as well as in the control group, plants with RFLP bands of sizes deviating from those in the *A. thaliana* parent were found. The bands of aberrant sizes might indicate the presence of intergenomic translocations. However, more likely explanations are intragenomic rearrangements or changes in methylation at *Eco*RI restriction sites. *Eco*RI was used in the DNA analysis of the hybrids and has been shown to be methylation-sensitive in some cases (Jen-Jacobson et al. 1996).

The effects of the different treatments on intergenomic translocation still remains to be investigated. Such a study would be of great interest to perform on the remaining plants to elucidate the optimal treatment and dose for introgression events. Nevertheless, this study clearly shows that the outsorting of DNA in asymmetric somatic hybrids is dose-dependent using UV- or X-irradiation. It also reveals that it is possible to get highly asymmetric hybrids using high irradiation doses.

Acknowledgements We thank Kristin-Sophie Mellsjö, Inga Blohm and Bertil Blohm for the practical work concerning the fertility investigation and the maintenance of the plants in the greenhouse. This work was granted by the Swedish Environmental Protection Agency, the Swedish Council for Forestry and Agricultural Research and the Nilsson-Ehle foundation, Sweden.

References

Banks MS, Evans PK (1976) A comparison of the isolation and culture of mesophyll protoplasts from several *Nicotiana* species and their hybrids. Plant Sci Lett 7:409–416

Bates GW, Hasenkampf CA, Contolini CL, Piastuch WC (1987) Asymmetric hybridization in *Nicotiana* by fusion of irradiated protoplasts. Theor Appl Genet 74:718–726

Bryant PE (1984) Enzymatic restriction of mammalian cell DNA using Pvu II and Bam H1: evidence for the double-strand break origin of chromosomal aberrations. Int J Radiat Biol 46: 57–65

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

Costa ND, Bryant PE (1990) The induction of DNA double-strand breaks in CHO cells by PvuII: Kinetics using neutral filter elution (pH 9.6). Int J Radiat Biol 57 : 933–938

Costa ND, Bryant PE (1991) Differences in accumulation of blunt- and cohesive-ended double-strand breaks generated by restriction endonucleases in electroporated CHO cells. Mutat Res 254 : 239–246

Costa ND, Masson WK, Thacker J (1993) The effectiveness of restriction endonucleases in cell killing and mutation. Som Cell Mol Gen 19 : 479–490

Dean C, Schmidt R (1995) Plant genomes: a current molecular description. Annu Rev Plant Physiol Plant Mol Biol 46 : 395–418

Dixon WJ, Massey FJ Jr (1969) Introduction to statistical analysis. McGraw-Hill, New York San Francisco St. Louis Toronto London, Sydney

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

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 (1998) UV dose-dependent DNA elimination in asymmetric somatic hybrids between *Brassica napus* and *Arabidopsis thaliana*. Plant Sci 131 : 65–76

Hinnisdaels S, Mouras A, Salesses G, Veuskens J, Taylor C, Gharti-Chhetri GB, Negrutiu I, Jacobs M (1992) Translocation events demonstrated by molecular, *in situ* hybridization and chromosome pairing analyses in highly asymmetric somatic hybrid plants. Transgenic Res 1 : 170–176

Hinnisdaels S, Jacobs M, Negrutiu I (1994) Asymmetric somatic hybrids. In: Bajaj YPS (eds) Somatic hybridization in crop improvement I. Springer, Berlin Heidelberg New York, pp 57–71

Jazdzewska E, Niklas A, Majewska-Sawka A (1995) Progress towards sugar beet improvement through somatic hybridization. Acta Soc Bot Pol 64 : 341–347

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

Lehmann EL (1975) Nonparametrics. Holden-Day, McGraw-Hill, San Francisco

Liu Y-G, Mitsukawa N, Lister C, Dean C, Whittier 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

Moses SAM, Christie AF, Bryant PE (1990) Clastogenicity of *Pvu*II and *Eco*RI in electroporated CHO cells asseyed by metaphase chromosomal aberrtions and by micronuclei using the cytokinesis-block technique. Mutagenesis 5 : 599–603

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

Natarajan AT, Obe G (1984) Molecular mechanisms involved in the production of chromosomal aberrations. III. Restriction endonucleases. Chromosoma 90 : 120–127

Parokonny AS, Kenton AY, Gleba YY, Bennett MD (1992) Genome reorganization in *Nicotiana* asymmetric somatic hybrids analysed by *in situ* hybridization. Plant J 2 : 863–874

Piastuch WC, Bates GW (1990) Chromosomal analysis of *Nicotiana* asymmetric somatic 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

Schmidt R, West J, Love K, Lenehan Z, Lister C, Thompson H, Bouchez D, Dean C (1995) Physical map and organisation of *Arabidopsis thaliana* chromosome 4. Science 270 : 480–483

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

Sjödin C, Glimelius K (1989) Transfer of resistance against *Phoma lingam* to *Brassica napus* by asymmetric somatic hybridization combined with toxin selection. Theor Appl Genet 78 : 513–520

Skarzhinskaya M, Fahleson J, Glimelius K, Mouras A (1998) Genome organization of *Brassica napus*, *Lesquerella fendleri* and GISH analysis of their somatic hybrids. Genome (in press)

Stoilov LM, Mirkova VN, Dimitrova A, Uzunova V, Gecheff KI (1996) Restriction endonucleases induce chromosomal aberrations in barley. Mutagenesis 11 : 119–123

Subrahmanyam NC, Gould AR, Doy CH (1976) Cleavage of plant chromosomes by restriction endonucleases. Plant Sci Lett 6 : 203–208

Thacker J (1994) The study of responses to 'model' DNA breaks induces by restriction endonucleases in cells and cell-free systems: achievements and difficulties. Int J Radiat Biol 66 : 591–596

Thompson CJ, Movva NR, Tizard R, Crameri R, Davies JE, Lauwereys M, Botterman J (1987) Characterization of the herbicide-resistance gene *bar* from *Streptomyces hygroscopicus*. EMBO J 6 : 2519–2523

Vlahova M, Hinnisdaels S, Frulleux F, Claeys M, Atanassov A, Jacobs M (1997) UV irradiation as a tool for obtaining asymmetric somatic hybrids between *Nicotiana plumbaginifolia* and *Lycopersicon esculentum*. Theor Appl Genet 94 : 184–191

Waara S, Glimelius K (1995) The potential of somatic hybridization in crop breeding. Euphytica 85 : 217–255

Winegar RA, Phillips JW, Youngblom JH, Morgan WF (1989) Cell electroporation is a highly efficient method for introducing restriction endonucleases into cells. Mutat Res 225 : 49–53

Zachgo EA, Wang ML, Dewdney J, Bouchez D, Camilleri C, Belmonte S, Huang L, Dolan M, Goodman HM (1996) A physical map of chromosome 2 of *Arabidopsis thaliana*. Genome Res 6 : 19–25