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Characterization of backcross generations obtained under field conditions from oilseed rape-wild radish F_1 interspecific hybrids: an assessment of transgene dispersal

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Abstract Gene flow from glufosinate-resistant transgenic oilseed rape to wild radish was studied over two backcross generations. Under field conditions, seed production from oilseed rape-wild radish F_1 hybrids due to pollination by wild radish was always low: on average 0.12 and 0.78 seeds per 100 flowers and per plant, respectively. The cytogenetics of the resulting $\ll BC_1 \gg$ plants can be explained in the main by three different genomic constitutions: either ACRrRr, 2n = 37, ACRr, 2n = 28 (the same chromosome number as the mother plant), or by the amphidiploid AACCRrRr, 2n = 56. The probability of gene exchange through chromosome pairing was high only in plants with 2n = 28 or 37 chromosomes. Due to the viability of unreduced or partially reduced female gametes, most of the $\ll BC_1 \gg$ plants (81.9%) were Basta resistant whereas the analysis of oilseed rape specific loci indicated that their transmission varied with the locus. In spite of low male fertility (8.7%), an improvement of the female fertility over the F_1 hybrids was observed with an average production of 1.4 and 11 seeds per 100 flowers and per plant, respectively. At the following $\ll BC_2 \gg$ generation, the *bar* gene transmission (57.2% of Basta-resistant plants) decreased as did the chromosome number, with a majority of plants having between 24 and 27 chromosomes, with 10.5% similar to wild radish (2n = 18). The lower the chromosome number, the better the fertility of the $\ll BC_2 \gg$ plants. On average, 7.9 and 229.3 seeds per 100 flowers and per plant were produced. Gene-flow assessment is discussed based on these data.

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Introduction

The risk of transgene flow from cultivated species to natural populations depends on the floral biology of the crop and on the presence within the cultivated areas of related weedy species. Genetically modified oilseed rape (*Brassica napus* L.), which has already been commercialized in North America, is a good model for gene-flow analysis within natural populations as it is a partially allogamous species with a long-distance pollen dispersal (Crawley et al. 1993).

Wild populations of oilseed rape, which is an amphidiploid (AACC, 2n = 38), have never been described. However, numerous cruciferous diploid weeds are present within, or close to, the oilseed rape fields and have a flowering period corresponding to that of the crop.

Several steps have to be overcome for efficient gene flow between species under field conditions including: (1) the production of viable F_1 interspecific hybrids, (2) the occurrence of fertile successive backcross generations, (3) gene transmission through the different generations, (4) effective gene introgression through recombination between the genomes, and (5) maintenance within the natural populations of the introgressed genes.

Numerous oilseed rape-wild species F_1 interspecific hybrids have already been produced either by hand pollination, followed sometimes by embryo rescue (Kerlan et al. 1992; Scheffler and Dale 1994), or after open pollination (Bing et al. 1991; Eber et al. 1994; Scheffler and Dale 1994; Chèvre et al. 1996). However, the production of the following generation by backcrossing is generally difficult (Bing et al. 1991; Chèvre

et al. 1996), and the return to the original diploid level of the weed under natural conditions has only been reported once, in *B. napus*-*B. campestris* F_1 hybrids (Mikkelsen et al. 1996). These authors used as the wild species *B. campestris* L. (syn. *B. rapa* L., AA, 2n = 20), which is one of the progenitors of oilseed rape, and only two backcrosses were needed to isolate fertile wild *B. campestris* plants containing a transgene from the *B. napus* genome. Among the more common oilseed rape weeds, we chose three species belonging to another genus, wild mustard (*Sinapis arvensis* L., SarSar, 2n = 18), hoary mustard (*Hirschfeldia incana* (L.) Lagrèze-Fossat, AdAd, 2n = 14) and wild radish (*Raphanus raphanistrum* L., RrRr, 2n = 18). Field experiments, using a cytoplasmic male-sterile oilseed rape variety as female parent and the three weeds as pollinators, revealed that interspecific F_1 hybrids were produced for all the combinations tested (Eber et al. 1994; Chèvre et al. 1996); but their frequency was highest between oilseed rape and wild radish and varied with the female oilseed rape genotype (Baranger et al. 1995). It was reported previously that chromosome pairing occurred between oilseed rape and wild radish genomes allowing gene exchange (Kerlan et al. 1993). Therefore, oilseed rape-wild radish was chosen as the best combination to analyse the genetic mechanisms involved in intergeneric gene flow. The characteristics of successive backcross generations grown under field conditions using wild radish as pollinator are presented and discussed.

Materials and methods

Plant materials

Oilseed rape-wild radish F_1 interspecific hybrids were produced under field conditions by Baranger et al. (1995) using six Ogu-INRA cytoplasmic male-sterile oilseed rape varieties as mother plants: three male-sterile lines, namely 'Miyuki' (Asian spring type), 'Drakkar' (European spring type), 'Samouraï' (European winter type), and three F_1 hybrids obtained by crossing each of the three male-sterile lines with a single transgenic line, 'Westar T5' (WT5) (Canadian spring type). WT5 contains a single copy of the *bar* gene which confers resistance to a broad-range herbicide, glufosinate-ammonium (commercial name, Basta), and was kindly provided by Plant Genetic Systems (Gent, Belgium).

A mixture of two wild radish populations, one collected in Dijon, kindly provided by Dr. H. Darmency (INRA, Dijon France) and the other in Rennes (France), was employed in all the field experiments as pollinators.

The scheme used to obtain the different $\ll BC_1 \gg$ and $\ll BC_2 \gg$ generations is presented in Fig. 1. As all the seeds were obtained by open pollination, some caution was used to describe the progeny and quotation marks were given to assign the different generations.

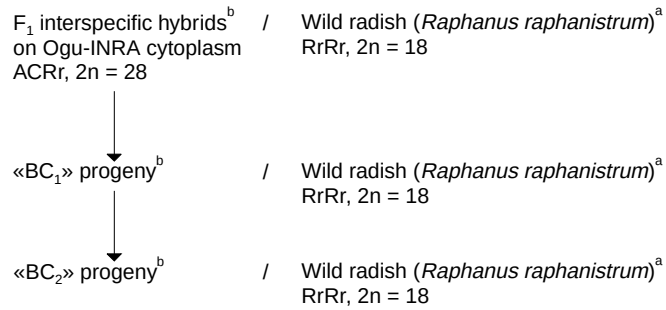

Fig. 1 Successive backcross generations analysed from F_1 interspecific hybrids obtained by a cross under field conditions between oilseed rape (AACC, 2n = 38) and wild radish (RrRr, 2n = 18)

Field design

Germination was carried out in Petri dishes and seedlings were transplanted in the greenhouse. At the 4–5 leaf stage they were vernalized at 4°C and then planted out in the field. All the field trials were surrounded with a 6-m band of wild radish to prevent pollen dispersal and were spatially isolated from any other oilseed rape field by at least 500 m. A ratio of 1 mother-plant : 1 wild radish plant, as pollinator, was always respected.

The field design was as described by Baranger et al. (1995) for the production of the first generation of backcross ($\ll BC_1 \gg$) seeds: within each of the five randomised blocks, the six plots consisted each of three rows of one genotype of F_1 interspecific hybrids and of three wild radish rows. The experiment was maintained over 2 years.

Different field experiments were performed according to the genomic structure of the $\ll BC_1 \gg$ plants and the number of hybrids available: either in an open field, for hybrids with 2n = 37 or 2n = 56 chromosomes, or under cages for the three other plant categories, i.e. those with 2n = 24–36, 38–55, or 57–85 chromosomes.

Only the Basta-resistant $\ll BC_2 \gg$ hybrids and the plants showing a chromosome number close to that of wild radish (i.e. 2n = 18) were analysed, using a single field trial as described for the production of $\ll BC_1 \gg$ seeds.

Field observations

The female fertility was assessed under field conditions by counting the number of pods per 50 flowers and the number of seeds per maximum 50 pods for each hybrid plant. From these data, the number of seeds/100 flowers was calculated. In addition, all the developed pods were harvested to calculate the total number of seeds per plant.

Basta treatment for testing the presence of the *bar* gene

Seedlings at the four-leaf stage were sprayed with a 1.2% Basta LS (200 g of glufosinate/l) + 0.1% SDS solution. The number of resistant and susceptible plants was scored after 10 days.

Cytogenetic studies

Chromosome counts were made from root meristems as described by Eber et al. (1994) and from leaves by quantitative DNA estimates using a flow cytometer (Partec Ploidy Analyser, Münster, Germany) according to Eber et al. (1997). The linear regression, obtained from

plants analysed by these two methods (Eber et al. 1997), enabled us to rely on the faster flow-cytometric method to determine the chromosome numbers of hybrids.

For meiotic analyses, floral buds were prepared as described by Eber et al. (1994).

Male fertility was assessed as the percentage of pollen stained by a 1% aceto-carmine solution. Two or three flowers and at least 600 pollen grains were analyzed per plant.

Isozyme analysis

Young leaves were crushed in a 0.1 M Tris-HCl buffer containing 1% reduced glutathione. Three isozyme systems selected from previous studies (Baranger 1995), phosphoglucomutase (PGM), phosphoglucoisomerase (PGI) and leucine amino-peptidase (LAP), were examined using the standard starch-electrophoresis method described by Chèvre et al. (1995). The staining procedures and the locus nomenclature for the A or C genomes of oilseed rape were as reported by Chèvre et al. (1995).

RAPD analysis

DNA was extracted from young leaves according to the method of Doyle and Doyle (1990), and using 12.5 ng of DNA the procedures were as described by Hu and Quiros (1991). The *Taq* polymerase was purchased from Eurobio (Les Ulis, France) and the random 10-mer primers from Operon Technologies (Alemada, Calif., USA). Three of them, OPA09, OPA16 and OPC13, were selected from previous studies (Baranger 1995). The samples were run on a 1.8% agarose gel at 3 V/cm to separate amplified products, which were visualized by staining with ethidium bromide (0.5 µg/ml). The loci are designated by an OP prefix followed by the kit letter, the primer number, and the size of the band (in base pairs).

Statistical analysis

The results were analysed on SAS software (Statistical Analysis System, SAS Institute Inc. 1989). When residues showed a normal distribution, an analysis of variance was used, and the different genotypes or chromosome-number classes were classified using the Student-Newman-Keuls test (SNK test, $\alpha = 0.05$). In the absence of a normal distribution, the non-parametric tests of Kolmogorov-Smirnov, in the case of two populations, or alternatively of Kruskall-Wallis in other cases, were employed.

The correlation coefficient and its significance level ($\alpha = 0.05$) for quantitative data were calculated by Kendall analysis.

Results

Production of $\ll BC_1 \gg$ seeds

The seed production of the F_1 interspecific hybrids (ACRr, 2n = 28) was always low (Table 1). The mean number of seeds per 100 flowers and per plant were 0.12 and 0.78, respectively. No significant year effect ($P > 0.05$) was detected. However, the analysis of variance revealed that the interspecific hybrids having as an oilseed rape mother plant an F_1 hybrid variety ('MiyukiWT5', 'DrakkarWT5' or 'Samouraï WT5') produced significantly more seeds per 100 flowers or per plant ($P < 0.05$) than the ones which had a pure line ('Miyuki', 'Drakkar' or 'Samouraï') as the mother plant.

Characterisation of the $\ll BC_1 \gg$ plants

Genomic structure

From a total of 1216 $\ll BC_1 \gg$ seeds, 74.2% germinated. Cytological results were obtained from 462 plants analysed either by mitotic chromosome counts and/or by flow cytometric analysis. Only 49.8% of the $\ll BC_1 \gg$ plants had 37 chromosomes with an expected ACRrRr genomic constitution, explained by an unreduced gamete of the interspecific hybrid mother plant (ACRr, 2n = 28) plus reduced gametes of the wild radish pollinator (Rr, n = 9). Among the other $\ll BC_1 \gg$ plants, 15.6% carried less than 37 chromosomes and these were mainly plants with the same chromosome number as the mother plants, i.e. 2n = 28. The remaining $\ll BC_1 \gg$ plants (34.6%) contained more than 37 chromosomes in which 2n = 56 with the expected genomic constitution AACCRrRr corresponded to the

Table 1 Production of $\ll BC_1 \gg$ seeds from F_1 interspecific hybrids

Mother-plants F_1 interspecific hybrids	1994			1995		
	No. of plants	No. of seeds/ 100fl	No. of seeds/ plant	No. of plants	No. of seeds/ 100fl	No. of seeds/ plant
'Miyuki'-Rr[a]	85	0.13	0.59	146	0.10	0.45
'Drakkar'-Rr	200	0.09	0.47	149	0.07	0.48
'Samouraï'-Rr	191	0.04	0.25	144	0.06	0.42
'MiyukiWT5'[b]-Rr	224	0.14	0.84	148	0.25	1.26
'DrakkarWT5'-Rr	351	0.08	0.42	151	0.14	1.65
'SamouraïWT5'-Rr	287	0.14	1.09	142	0.22	1.39

[a]Rr: *Raphanus raphanistrum*
[b]WT5: var. 'Westar' containing the *bar* gene

Table 2 Meiotic behaviour of the $\ll BC_1 \gg$ plants

2n	No. of plants	No. of cells	Meiotic behaviour			
			Univalents	Bivalents	Trivalents	Quadrivalents
28	7	94	9.9 (6–14)*	8.96 (6–11)	0.02 (0–1)	0.02 (0–1)
37	57	937	4.10 (0–17)	15.97 (10–18)	0.15 (0–2)	0.13 (0–2)
56	2	24	15.71 (12–20)	19.67 (18–22)	0.21 (0–2)	0.08 (0–1)
56	5	48	7.23 (2–13)	23.98 (20–27)	0.10 (0–1)	0.13 (0–1)

* Ranges are indicated in parenthesis

main class. The same chromosome categories and distribution were observed irrespective of the initial genotype of the F_1 interspecific hybrid mother plants.

Chromosome pairing in plants with $2n = 28$, $2n = 37$ and $2n = 56$ chromosomes was analyzed (Table 2). Plants with $2n = 28$ chromosomes showed a high frequency of chromosome pairing, mainly as bivalents. In the $\ll BC_1 \gg$ plants with $2n = 37$ chromosomes (ACRrRr) the two wild radish genomes would be expected to form nine bivalents but additional chromosomes formed pairing partnerships as bivalents or multivalents. The homologous genomes of the amphidiploids (AACCRrRr, $2n = 56$) showed a lower frequency of chromosome pairing than anticipated from the results obtained in other crucifer species. Several chromosomes remained as univalents and, according to the frequency of chromosome pairing, two classes could be distinguished.

Inheritance of oilseed rape loci

All the seedlings obtained from herbicide-resistant F_1 interspecific hybrids, which contained one copy of the *bar* gene, were sprayed with Basta solution. Among the 674 plants tested, 81.9% were resistant indicating a high level of *bar* gene transmission from F_1 hybrids to the $\ll BC_1 \gg$.

In order to study other oilseed rape loci, isozyme and RAPD analyses were performed. Initially, we analysed the genetic variability of the wild radish populations and of the oilseed rape varieties used to produce the interspecific hybrids. For the three isozyme systems examined, ten isozyme loci were identified from the oilseed rape varieties employed and five from wild radish, respectively. Taking into account the combined allelic variation of the wild radish populations, only seven distinct oilseed rape loci always carried allelic variants with a mobility different from that of any wild radish genotype. These were *Pgi-2C, Lap-1A, Lap-1C, Pgm-1A, Pgm-1C, Pgm-2A, Pgm-3A* and they were used to estimate the transmission of oilseed rape loci in $\ll BC_1 \gg$ plants. In the same way, the profiles obtained from three DNA bulks of ten plants from each of the two wild radish populations were compared with the profiles obtained for the different oilseed rape varieties, using three primers. Ten oilseed rape dominant markers were identified in at least one of the oilseed rape varieties used, but were always absent in the wild radish bulks.

Using these markers and the *bar* gene, 43 $\ll BC_1 \gg$ plants were analysed (16, 22, and 5 plants with $2n < 37$, $2n = 37$, and $2n > 37$, respectively). The only significant difference ($P < 0.05$) for the transmission of oilseed rape markers was between plants with $2n < 37$ (72.2%) and plants with $2n > 37$ (87.5%). From the plants with 37 chromosomes, 82.7% of the oilseed rape loci were transmitted.

The transmission of oilseed rape loci was analysed in a population of 21 plants ($2n = 37$) produced from one interspecific hybrid plant ('Samouraï WT5'-Rr, ACRr, $2n = 28$). For example, the profiles obtained from OPA09 (Fig. 2) revealed two specific oilseed rape loci (OPA09-700 and OPA09-1000). Among the 17 oilseed rape loci studied including the *bar* gene, the percentage of transmission varied according to the locus and ranged from 47.6% (OPA16-1400) to 100% (*bar* gene, *Lap-1A* or *Pgm-3A*) (Fig. 3).

Fertility

Different field trials were performed according to the genomic structure of the plants and the number of hybrids available: three independent cages for plants with a chromosome number ranging from 24 to 36, 38 to 55 or 57 to 85, respectively, and two isolated fields for hybrids with $2n = 37$ or $2n = 56$ chromosomes. Most of the plants having 37 chromosomes or less had a morphology very close to that of wild radish.

The results concerning male fertility are reported in Table 3. Despite an increase of the percentage of

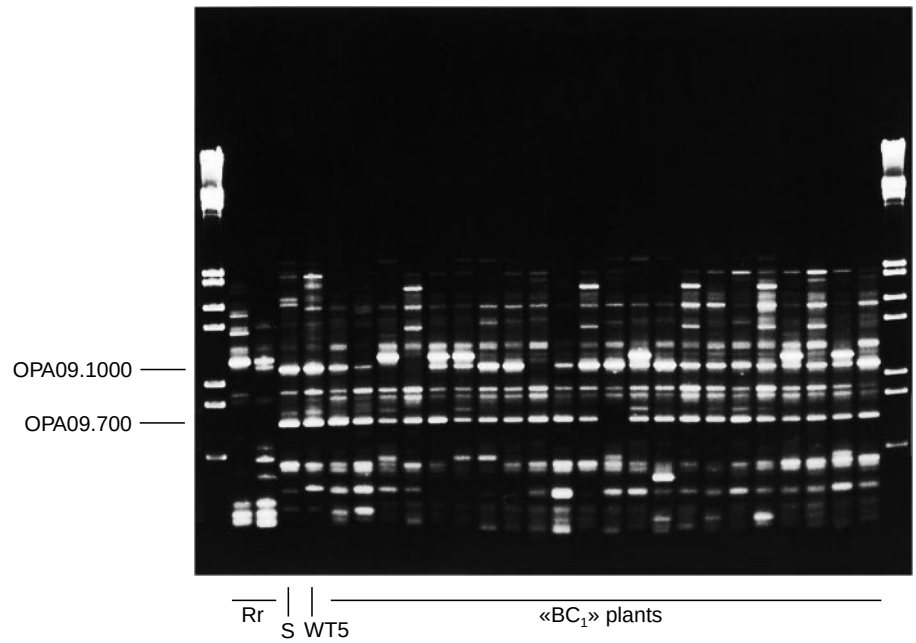

Fig. 2 Amplification profiles obtained with the OPA09 primer from DNA of two bulks of wild radish (Rr), two oilseed rape varieties, 'Samouraï' (*S*) and 'WestarT5' (*WT5*), and $\ll BC_1 \gg$ plants (2n = 37) obtained from one F_1 interspecific hybrid. Deletion of two specific oilseed rape loci, OPA09.700 and OPA09.1000, was observed

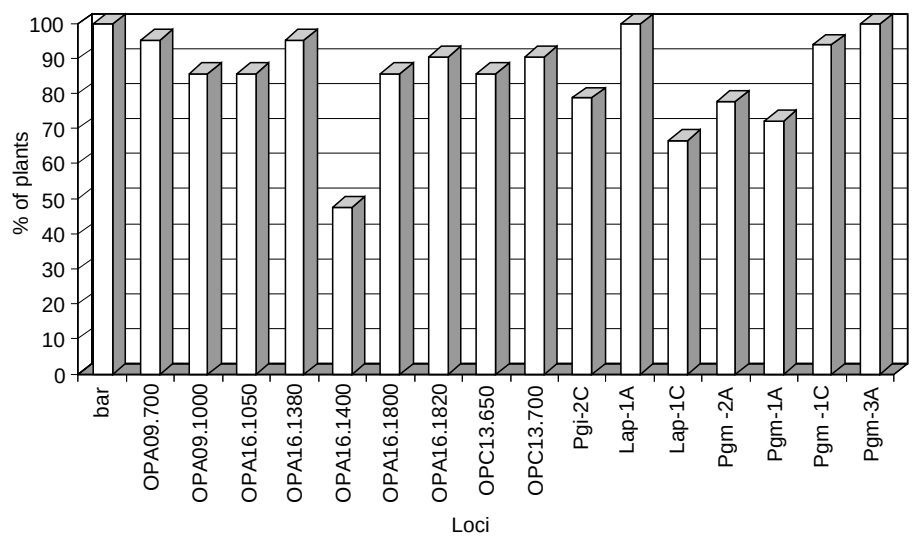

Fig. 3 Percentages of $\ll BC_1 \gg$ plants (2n = 37) obtained from one F_1 interspecific hybrid presenting different specific oilseed rape loci, the *bar* gene, isozymes and RAPDs

male-fertile plants with increasing chromosome number, no significant difference was revealed between the different genomic categories ($\chi 2 = 5.19$, $P < 0.05$).

In contrast, non-parametric tests indicated that the differences for female fertility, expressed by the number of seeds/100 flowers or per plant (Table 3), were significant between the different genomic classes. The lower the chromosome number, the better the female fertility. In a like manner, the percentages of plants with at least one seed increased with a decrease in the chromosome number. However, the inter-plant variation in the same class was high.

Characterisation of the $\ll BC_2 \gg$ plants

Only seeds harvested on Basta-resistant $\ll BC_1 \gg$ plants with 2n = 28, 37 or 56 chromosomes were studied. From a total of 1401 $\ll BC_2 \gg$ seeds, 87% germinated.

Genomic structure

The chromosome number was determined either by chromosome counts and/or by flow-cytometer analyses

Table 3 Male and female fertility of the $\ll BC_1 \gg$ plants according to their genomic structure

Fertility		Chromosome number				
		(24–36)	37	(38–55)	56	(57–85)
Male	No. of plants	35	168	32	63	30
	% Fertility	2.9	8.1	17.5	9.1	10.8
		(0–22.9)[a]	(0–68.3)	(0–57.0)	(0–51.3)	(0–51.3)
	% Fertile plants[b]	25.7	38.7	46.9	42.8	50
Female	No. of plants	37	169	36	67	28
	No. seeds/100 flowers	3.3	1.5	0.7	0.05	0.09
	No. seeds/plant	50.6	9.2	6.4	0.7	0.3
		(0–1059)	(0–256)	(0–129)	(0–14)	(0–5)
	% Plants with at least one seed	59.5	55.0	38.9	16.4	14.3

[a] Ranges are indicated in parenthesis
[b] Plants with at least some aceto-carmine stained pollen grains

on 1083 $\ll BC_2 \gg$ plants. The majority of the plants (66.9%) had between 22 and 28 chromosomes and 10.5% had the same chromosome number as the wild radish, i.e. $2n = 18$. However, from 272 $\ll BC_2 \gg$ plants derived from $2n = 28$ $\ll BC_1 \gg$ plants, a majority (38.6%) had 18 chromosomes and most of the others between 20 and 25 chromosomes (overall 41.2%). The decrease in chromosome number was lower for the 795 $\ll BC_2 \gg$ plants obtained from the $2n = 37$ $\ll BC_1 \gg$ hybrids: most of them (76.6%) showed between 23 and 28 chromosomes and only 1.1% had the same chromosome number as wild radish. Only 16 plants were obtained from the amphidiploids, and so no conclusion about the effect of the mother plant on the evolution of chromosome number was possible. However, they carried between 33 and 60 chromosomes.

Inheritance of oilseed rape loci

The transmission of the *bar* gene was analysed by spraying the $\ll BC_2 \gg$ plants with a Basta solution. Among the 1028 plants treated, 57.2% were Basta resistant. None of the plants having the same chromosome number ($2n = 18$) as the wild radish were resistant. The transmission rate was significantly higher (63.3%) for the 848 $\ll BC_2 \gg$ plants obtained from $2n = 37$ $\ll BC_1 \gg$ hybrids than for the 164 $\ll BC_2 \gg$ plants obtained from $2n = 28$ $\ll BC_1 \gg$ hybrids (28.1%). Even though the percentage of resistant plants produced from the amphidiploids was low (31.3%), too few plants were analysed to arrive at a decisive conclusion.

Fertility

All the Basta-resistant plants and the hybrids with a chromosome number ranging from 18 to 21 were grown in the same field. The majority of the $\ll BC_2 \gg$ hybrids were morphologically very similar to wild radish. However, the lower the chromosome number of the $\ll BC_2 \gg$, the more chlorophyll-deficient were the plants.

Compared to the previous $\ll BC_1 \gg$ generation, the male fertility was higher whatever the method of assessment: the percentage of pollen stained or of fertile plants (Table 4). The hybrids which showed the best percentage of fertility had a chromosome number close to that of wild radish. However, even though the correlation coefficient between chromosome number and male fertility was significant, it was low ($r = -0.19$).

The female fertility was also improved (Table 4) compared to that of the $\ll BC_1 \gg$ hybrids; 82.5% of the plants showed at least one seed and the plants with the lowest chromosome number had a female fertility similar to that of wild radish. The correlation analysis revealed that only the number of seeds per plant was significantly negatively correlated with the chromosome number ($r = -0.25$): the lower the chromosome number, the higher the female fertility.

Discussion

For the occurrence of transgene flow from a crop to a weed, the first step is the production of spontaneous F_1 interspecific hybrids. This has already been demonstrated between oilseed rape and different crucifer weeds (Bing et al. 1991; Eber et al. 1994; Jorgensen and Andersen 1994; Baranger et al. 1995; Chèvre et al. 1996). However, the stable introgression of transgenes within natural populations depends on the fertility, the genomic structure, and the transmission of oilseed rape genes within successive generations. In the present paper, from F_1 oilseed rape-wild radish interspecific

Table 4 Male and female fertility of the $\ll BC_2 \gg$ plants

Male fertility			Female fertility			
No. of plants	% Fertility	% Fertile plants[b]	No. of plants	No. seeds/ 100 flowers	No. seeds/plant	% Plants with at least one seed
452	46.2 (0–92.9)[a]	53.8	443	7.9	229.3 (0–5673)	82.5

[a] Ranges are indicated in parenthesis
[b] Plants with at least some aceto-carmine stained pollen grains

hybrids, we describe the $\ll BC_1 \gg$ and $\ll BC_2 \gg$ progeny obtained under field conditions, using wild radish as a pollinator.

The production of $\ll BC_1 \gg$ seeds was around 100-times lower than that obtained in the previous F_1 generation (Baranger et al. 1995). It is more difficult to produce the first backcross generation than the original F_1 interspecific hybrids because of the high level of sterility of these triploid plants. Because of the poor seed set, this step is likely to be difficult to overcome, but F_1 interspecific hybrid plants have a very long flowering period. We demonstrate that the genotype of oilseed rape has a very significant effect on the production of F_1 interspecific hybrids under field conditions (Baranger et al. 1995) and these results were confirmed during a second year of experimentation (data not shown). At the following $\ll BC_1 \gg$ generation, the F_1 interspecific hybrids which had a F_1 hybrid oilseed rape variety as mother plant produced more seeds than those which had an oilseed rape line as the mother plant. The vigour of the F_1 interspecific hybrids, which was very close to that of the original oilseed rape, might explain this result.

A large variation in the genomic structure of the $\ll BC_1 \gg$ plants was observed, irrespective of the original oilseed rape variety used. In our field conditions most of the $\ll BC_1 \gg$ hybrids had the expected BC_1 structure, explained by the pollination of female unreduced gametes (ACRr, $2n = 28$) by male-reduced wild radish pollen (Rr, $n = 9$). The production of unreduced gametes in Crucifer species was reported by Heyn (1977). Moreover Namai (1987) showed that, from amphihaploids, the frequency of univalents was correlated with the number of unreduced gametes. However, the analysis of oilseed rape-specific loci from $\ll BC_1 \gg$ ($2n = 37$) plants revealed that not all the oilseed rape loci were transmitted. A more precise study of $\ll BC_1 \gg$ plants, all obtained from the same F_1 interspecific mother plant, indicated that the transmission rate varied between different oilseed rape loci. Seven of the markers analysed were located on six different linkage groups of the oilseed rape genetic map initiated by Foisset et al. (1996). Studies are in progress to analyse this position effect from different transgenic lines and from specific oilseed rape loci. The non-reduction of the gametes is thought to occur after the first stage of meiotic chromosome pairing during which chromosome exchange takes place. As the genomic structure of the resulting plants remained the same, the gametes probably resulted from a first-division restitution (FDR), as described by Veilleux (1985). The meiotic behaviour of these expected ACRrRr ($2n = 37$) plants revealed a high level of chromosome pairing; it is likely that the homologous genomes of wild radish formed nine bivalents and that homoeologous pairing occurred between the A and C genomes of oilseed rape, with a maximum of three or five bivalents on average, as described previously by Renard and Dosba (1980). However, more chromosome pairing was observed in our study, especially in the form of multivalents, indicating interspecific chromosome pairing allowing gene flow from one genome to another.

In some cases, a partial gamete reduction probably took place since 15.6% of the $\ll BC_1 \gg$ hybrids had less than 37 chromosomes. This observation was confirmed by the high rate of oilseed rape locus deletion. If we hypothesize that wild radish remained the pollinator, the chromosome complement of the female gametes ranged from 15 to 27 chromosomes. The main $\ll BC_1 \gg$ category carried 28 chromosomes: these plants were either produced from partially reduced gametes with 19 chromosomes or were matromorphic. This last hypothesis was not supported by the meiotic behaviour, which revealed a higher frequency of chromosome pairing than that observed in the mother plants (Kerlan et al. 1993; Eber et al. 1994). However, we noted that some $\ll BC_2 \gg$ plants obtained from certain $2n = 28$ $\ll BC_1 \gg$ hybrids had the same chromosome number and morphology as the mother plant (data not shown) so that the presence of apomictic phenomena was not excluded.

Other $\ll BC_1 \gg$ plants showed more than 37 chromosomes. They were produced either from unreduced wild radish gametes (RrRr, $2n = 18$), especially in the case of plants with a chromosome number close to 46 (ACRr + RrRr), by inter-pollination of F_1 interspecific

hybrids since some of them were partially pollen-fertile (Baranger et al. 1995), or by polyploidisation before or after pollination. This last hypothesis was supported by the observation of plants with more than 56 chromosomes. Several of these genetic mechanisms were probably implicated. We expected a regular meiotic behaviour from amphidiploids (AACCRrRr) since all the genomes were at the diploid stage. In fact, the frequency of univalents was generally higher than that reported in some other amphidiploids containing the oilseed rape genome and the cabbage, wild mustard (Kerlan et al. 1993) or white mustard (Chèvre et al. 1994) genome.

The $\ll BC_1 \gg$ plants with the lowest chromosome number showed the highest level of female fertility and amphidiploids had a very low seed production. No correlation could be established between female and male fertilities. These data might be explained by the fact that the improvement of male fertility was dependant on the presence of a restorer gene(s) in the Ogu-INRA male-sterile system employed (Pelletier et al. 1983). Such restoration was most likely carried by the wild radish pollinator.

A general decrease of chromosome number was observed in the $\ll BC_2 \gg$ plants. This was linked to the structure of the mother plants; $\ll BC_1 \gg$ plants with the lowest chromosome number gave progeny with a ploidy level closer to that of wild radish, indicating that the partial reduction of the female gamete remained efficient in this generation. This observation was confirmed by the transmission rate of the *bar* gene which decreased with the chromosome number of the mother plant. Other specific oilseed rape loci will be studied in future. However, the closer the hybrids were to wild radish, the better was their fertility. In this case they were morphologically very similar to wild radish except for the chlorophyll deficiency. This character was initially described when an oilseed rape nucleus was introduced into Ogura cytoplasm and is the reason why Ogu-INRA male-sterile cybrids with oilseed rape chloroplasts were produced by protoplast fusion (Pelletier et al. 1983). So, reciprocally, it seems that the presence of oilseed rape chloroplasts with a radish nucleus induces a chlorophyll deficiency.

Several authors reported that when an allopolyploid, such as oilseed rape, is involved in interspecific crosses, it is generally difficult to return by backcrossing to a diploid level (Bing et al. 1991; Chèvre et al. 1996). The tendency in crucifer species is for spontaneous genome fixation by amphiploidy (Song et al. 1993). This is in disagreement with our observations which indicated a low probability of amphidiploid maintenance due to meiotic instability and poor seed set under natural conditions. Mikkelsen et al. (1996) reported that it is possible to obtain an introgressed weedy *B. campestris* (AA, 2n = 20), one of the progenitors of oilseed rape, after two backcrosses to *B. campestris* on F_1 AAC hybrids. Thus, if the transgene was located on the same oilseed rape genome as that of the weed, its transfer will be frequent. By contrast, the transfer of a transgene integrated into a C-genome chromosome will be difficult (Metz et al. 1997). Our data indicates that, from a species belonging to another genus (*R. raphanistrum*, RrRr, 2n = 18) than oilseed rape, the probability of obtaining a BC_1 generation from F_1 interspecific hybrids under field conditions was low, whereas it is possible to produce BC_2 fertile plants with a chromosome number close to that of the weed. However, the transgene transmission rate, which was high at the $\ll BC_1 \gg$ generation, decreased in $\ll BC_2 \gg$ plants. This same tendency was also observed at the following generation (Chèvre et al. 1997).

Taking into account the genetic variability of the two species concerned, we demonstrate that it is possible under field conditions to obtain herbicide-resistant plants close to wild radish in three generations. The germination rate was never a limiting factor. But no stable oilseed rape introgression within the wild radish genome was observed. The study of the following backcross generations will allow us to obtain information on the probability of this transfer. However, it is important to note that, reciprocally, for oilseed rape improvement, introgression of a radish restorer gene within the *B. napus* genome was obtained by Pellan-Delourme and Renard (1988).

Complementary analyses will be performed in future to assess the fitness of chlorophyll-deficient hybrids. The effect of the original cytoplasm will be assessed, under natural conditions, either from reciprocal hybrids already obtained (Darmency et al. 1995) or from male-fertile hybrids obtained in advanced backcross generations and used as pollinators on wild radish plants.

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