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Effect of oilseed rape genotype on the spontaneous hybridization rate with a weedy species: an assessment of transgene dispersal

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Abstract Spontaneous outcrossing of different male-sterile rapeseed lines and transgenic hybrids with a population of a weedy species, *Raphanus raphanistrum* L., has led to the harvest of numerous seeds showing a size dimorphism. Flow cytometry analysis correlated with chromosome counts showed that all of the large seeds belonged to rapeseed, whereas the small seeds were a mixture of mostly interspecific triploid hybrids, with some trigenomic amphidiploids, diploid and haploid rapeseed plants. Significant differences were revealed between the rapeseed lines and transgenic hybrids in their ability to form interspecific hybrids with *Raphanus raphanistrum* under natural conditions. Resistance to the herbicide Basta was properly expressed in the triploid and amphidiploid hybrids. Low male fertility of the interspecific triploid hybrids was not correlated with seed set in the subsequent generation.

Key words Transgenic male-sterile *Brassica napus* · Herbicide resistance · *Raphanus raphanistrum* · Interspecific hybridization · Flow cytometry

Introduction

Risk assessment of genetically modified crops prior to their release and commercialization has to focus on various issues. Among the basic concerns commonly referred to are the control of the size and detailed sequence of the DNA fragment that is transferred and the possible mutagenic effects that the transformation process can cause on the recipient genome. Ideally, the molecular biology techniques employed should ensure the selection of only single-copy transformants that do not carry any sequence of plasmid origin, but only a size-restricted T-DNA free of any bacterial origin of replication (Düring 1994) and free of any marker gene (Yoder and Goldsbrough 1994). In addition, field testing of transgenic lines leads to the elimination of at least the undesirable mutants for scored agronomic traits (De Greef et al. 1989; Dale et al. 1993). Another consideration to be taken into account is the possible toxicity of the new crop for its potential consumers (humans, cattle or pollinators), either owing to the encoded enzyme itself or to the products of the reaction it catalyzes (Kessler et al. 1992). A detailed analysis of the biochemical pathways a transgene can induce is therefore desirable (Dröge-Laser et al. 1994).

Even when these questions are fully answered, the main point still to be assessed is the possible dispersal of the transgene to other organisms belonging to other genera or species. Up to now, there has been no absolute evidence for horizontal transfer of the transgene to organisms belonging to other kingdoms, such as microorganisms involved in symbiosis or infection phenomena (Prins and Zadocks 1994), although some histological and DNA homology studies suggest that it might be possible (Bryngelsson et al. 1988). On the other hand, the levels and consequences of intraspecific transfers will very much depend on the dissemination mechanisms of the species, mainly pollination and seed dispersion, and on the competitiveness of the crop (Crawley et al. 1993). The potential of a transformed crop to become a weed itself can be looked at with the knowledge we already have on its dispersion, adding the potential presence of selection pressure.

An "intermediate" event would be the transfer of the transgene to related wild species through sexual hybridization. Wild species often occur as weeds in many crop fields, where they are difficult to get rid of owing to the frequent lack of selective herbicides. For some of these crops, such as beets (Boudry et al. 1993) and rapeseed (Eber et al. 1994; Jorgensen and Andersen 1994), it has been shown that interspecific hybrids can be formed naturally in the field.

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Interspecific and intergeneric hybridization within the *Cruciferae* has been intensely studied, either to deduce phylogenetic relationships (Mizushima 1980) or for breeding purposes, mostly with the aim of transferring the traits of interest from the wild species to the crop. These crosses were generally performed under controlled conditions. Indeed, species barriers in the *Cruciferae* can in some cases be technically overcome. As far as *Brassica napus* L. (AACC 2n = 38) is concerned, reported hybridizations with close relatives often imply manual pollination, embryo rescue (reviewed by Scheffler and Dale 1994) or somatic hybridization (Glimelius et al. 1991). There are very few reports of spontaneous crosses with relatives under natural conditions (Jorgensen and Andersen 1994; Scheffler and Dale 1994). Intergeneric crosses between *B. napus* and *Raphanus sativus* RR 2n = 18 (Takeshita et al. 1980; Lelivelt and Krens 1992; Rosén and Olin-Fatih 1993) or *Raphanus raphanistrum* RrRr 2n = 18 (Kerlan et al. 1992) have been successfully accomplished using embryo rescue or somatic hybridization. More recently, spontaneous hybridization of a male-sterile rapeseed cultivar with the wild species *Brassica adpressa* and *Raphanus raphanistrum* has been reported (Eber et al. 1994). These trials used a single non-transformed rapeseed cultivar, 'Brutor', to produce interspecific hybrids. The present paper deals with the relative potential of different rapeseed lines and transgenic hybrids to hybridize with *R. raphanistrum* on a large-scale trial and with the main features of the resulting interspecific hybrids.

Materials and methods

Plant materials

A single transgenic line 'Westar T5', kindly provided by Plant Genetic Systems (Gent, Belgium), was used in all the preliminary crosses. The transformation protocol of rapeseed hypocotyl fragments is described in De Block et al. (1989) and used the binary *Agrobacterium* vector C58C1 Rif^R (pMP90) (PGSFR781 A) described in De Block (1990). The transgenic line therefore carried the *bar* coding sequence conferring resistance to a broad-range herbicide, glufosinate (Thompson et al. 1987), known commercially as Basta, under the control of the CaMV 35S promoter and the termination and polyadenylation signals of the octopine T-DNA gene 7 (Velten and Schell 1985). Construction of the *bar* cassette is described in De Block et al. (1987).

The transgenic line 'Westar T5' was testcrossed for heterozygosity and copy-number evaluation to a susceptible non-transgenic 'Westar' line.

Different cytoplasmic male-sterile lines, namely 'Brutor', 'Drakkar' (European spring types), 'Miyuki' (Asiatic spring type), 'Samourai' (winter type) and 'Hobson' (winter forage synthetic type), all carrying the recombinant 'Ogura' cytoplasm (Pelletier et al. 1983), were used as female recipients in a topcross with 'Westar T5'. This resulted in a set of five F_1 hybrid rapeseed genotypes, all being cytoplasmic male-sterile ('Westar T5' carried no restorer gene), and heterozygous for the *bar* gene. These five genotypes, together with the five parental male-sterile lines as controls, were used as females.

Pollination was ensured by growing plants from seeds of a locally collected (Brittany, France) population of wild radish (*R. raphanistrum*).

Field design

Oilseed rape seeds were put to germinate in the greenhouse, vernalized in a cold room (4 °C, for 3 weeks for spring types and 8 weeks for winter types) and then planted out in the field at the four- to six-leaf stage. The trial was spatially isolated from any other rapeseed field by at least 500 m. The design consisted of plots of three *R. raphanistrum* rows 2 m long alternating with rapeseed plots of three rows 2 m long of each rapeseed genotype, in a completely randomized experiment consisting of five blocks. Each block included the ten genotypes being considered without any intrablock replication. In order to improve the pollen pressure from the wild species, the trial was surrounded by a *R. raphanistrum* 6-m-wide border.

In a second-year experiment, triploid hybrids were planted out in the field using the same design, and putative BC_1 seeds were harvested from the hybrids.

Field observations and harvest procedures

Flowering stages were followed according to the specifications proposed by the Groupe de travail Cetiom-Inra-PV (Leterme 1988).

Harvest took place only on the rapeseed male-sterile recipients. Twenty plants were sampled individually per genotype per block. Seed set was assessed on the primary stem as follows: firstly, the number of pods per 50 pollinated flowers was counted, then the number of seeds coming from 50 pods. We inferred from these two results the number of seeds from 100 pollinated flowers by the formula: number of pods /100 pollinated flowers × number of seeds per pod, from which we later deduced the number of hybrid seeds per 100 pollinated flowers (see below). The total number of seeds from the whole harvest was then evaluated, as well as the number of hybrid seeds per plant.

Seeds were sieved into a 1.6-mm sifter, previous experiments having shown that interspecific hybrids originate from smaller seeds (Eber et al. 1994). Large as well as small seeds were germinated in petri dishes at room temperature, and germination percentages were determined for at least 400 seeds harvested from the parental rapeseed lines and for at least 800 seeds harvested from the parental rapeseed hybrids.

Bar gene copy number

Total DNA extractions were performed according to Hu and Quiros (1991) followed by a phenol chloroform purification. Southern blots were performed according to the commonly used molecular techniques (Sambrook et al. 1989). Total DNA was digested by *Nco*I and *Eco*RV independently, fractioned on 1% agarose gels, transferred to nylon Hybond N+ membranes and hybridized with multiprime labeled DNA. Two probes were used to check for copy numbers. The right border was checked with a 550-bp internal fragment of the *bar* coding sequence (*bar* probe). After washing the filters, a second probe of 1,027 bp of T-DNA was used to check for the left border (T-DNA probe). Probes were kindly provided by PGS. These two fragments cover sequences on both sides of unique restriction sites for *Nco*I and *Eco*RV, and therefore a single band per insertion site was expected on Southern blots after digestion by either one of these two enzymes.

Phenotypical expression of the bar gene

Resistance to glufosinate was scored either by spraying at the four-leaf stage with a 1.2% Basta LS + 0.1% SDS solution, or by a non-destructive foliar resistance test using a 1% Basta LS + 0.1% SDS solution deposited on a limited leaf surface.

Cytogenetics

Chromosome counts were made on root-tip dividing cells according to Eber et al. (1994) or by quantitative DNA estimates using a flow cytometer (Partec Cell Analyzer, Chemunex, France). Small leaf

extracts were macerated with a razor blade in 2 ml of a nuclei extraction solution together with 20 µl of a chemiluminescent compound (respectively, Ploidy Buffer and Ploidy Dye purchased from Chemunex, France). Pea leaf extracts were used as internal standards.

Pollen fertility was estimated at first by counting acetocarmine-stained pollen grains (600 pollen grains per plant), then visually using three classes, namely class 1 for sterile plants, class 2 for a fertility up to 10% and class 3 when fertility ranged from 10% to 30%.

Statistical analysis

Analysis of variance was performed on the SAS/STAT version 6.07 Software using the GLM procedure. The mean number of hybrid seeds/100 pollinated flowers was surveyed in a factorial experiment that included three factors:

- factor no. 1: the original genetic background, with five levels including 'Brutor', 'Drakkar', 'Miyuki', 'Samourai', 'Hobson'
- factor no. 2: the hybrid state, either crossed with 'Westar T5' or not
- factor no. 3: blocks with five levels.

Results

Bar gene copy number and expression

Southern blots of total DNA digested by *Nco*I and *Eco*RV and probed consecutively with the *bar* and the T-DNA probes are shown on Fig. 1. As *Nco*I and *Eco*RV are single-site cutting enzymes in the T-DNA, the single bands obtained in all four cases showed that 'Westar T5' carried a single insertion site of the transgene.

On the other hand, Basta resistance tests on 80 test cross-derived seedlings did not show segregation for resistance. 'Westar T5' ('WT5') is therefore homozygous for the *bar* gene.

Preliminary spraying of the rapeseed hybrid genotypes 'Brutor' × 'Westar T5' (Bru × WT5), 'Drakkar' × 'Westar T5' (Dra × WT5), 'Miyuki' × 'Westar T5' (Miy × WT5), 'Samourai' × 'Westar T5' (Sam × WT5) and 'Hobson' × 'Westar T5' (Hob × WT5) with Basta disclosed that the *bar* gene in the heterozygous condition conferred resistant phenotypes for all of them (150 plants per genotype).

Production of interspecific hybrids

The flowering time of rapeseed and wild radish matched well, and as flowering in *Raphanus* is spread out over a very long period, the pollen pressure from the wild species was present during the whole of the rapeseed flowering period.

Seeds harvested from all the lines and hybrids displayed a size dimorphism above or below 1.6 mm in diameter (Table 1). The ratio of small seeds among the total number of seeds produced was quite variable, depending on the genotype, ranging from 10% to 83% of the total amount of seeds.

Investigation into the ploidy and chromosome numbers of small and large seeds was performed by mitotic counts and/or by flow cytometry. Sixty-one seedlings derived from large seeds were checked by both techniques with consistent results. An additional 183 plants were surveyed by either of the two techniques. On the whole, the 244 large-seed-derived seedlings equally sampled among the ten crosses all carried the typical rapeseed genomic constitution AACC of 38 chromosomes.

On the other hand, seeds with a diameter below 1.6 mm gave rise to plants that were variable in their ploidy and genomic constitution. Among the 240 plants studied, most were ACRr triploid hybrids with 28 chromosomes, but some AACCRrRr amphidiploids carrying 56 chromosomes were also obtained, as well as normal diploid AACC (38 chromosomes) and haploid AC (19 chromosomes) rapeseed plants. One hundred and thirty-two plants were surveyed both by mitotic counts and flow cytometry (Fig. 2), and in all cases the information obtained from the two techniques coincided.

Whatever the seed diameter, chromosome counts and flow cytometry gave consistent results even for

Fig. 1A, B Southern hybridizations of the transgenic line 'Westar T5' probed with the *bar* probe (**A**) and with the T-DNA probe (**B**). Ten micrograms of total genomic DNA were digested with *Eco*RV (**I**) or *Nco*I (**II**)

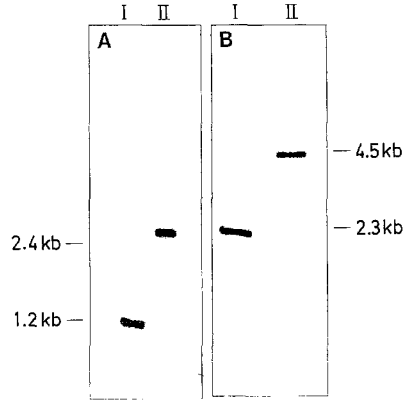

Table 1 Percentages of small and large seeds of five blocks harvested for the ten different female parental genotypes

Cross	Number of seeds	Percentage of seeds with a diameter	
		≤ 1.6 mm	> 1.6 mm
Bru × RrRr	10,400	14.6	85.4
Dra × RrRr	64,900	53.4	46.6
Miy × RrRr	3,600	10.2	89.8
Sam × RrRr	49,600	71.4	28.6
Hob × RrRr	40,900	83.9	16.1
{Bru × WT5} × RrRr	58,800	46.6	53.4
{Dra × WT5} × RrRr	70,300	30.1	69.9
{Miy × WT5} × RrRr	113,800	59.1	40.9
{Sam × WT5} × RrRr	116,700	60.3	39.7
{Hob × WT5} × RrRr	122,300	67.8	32.2
Total	651,400	57.6	42.4

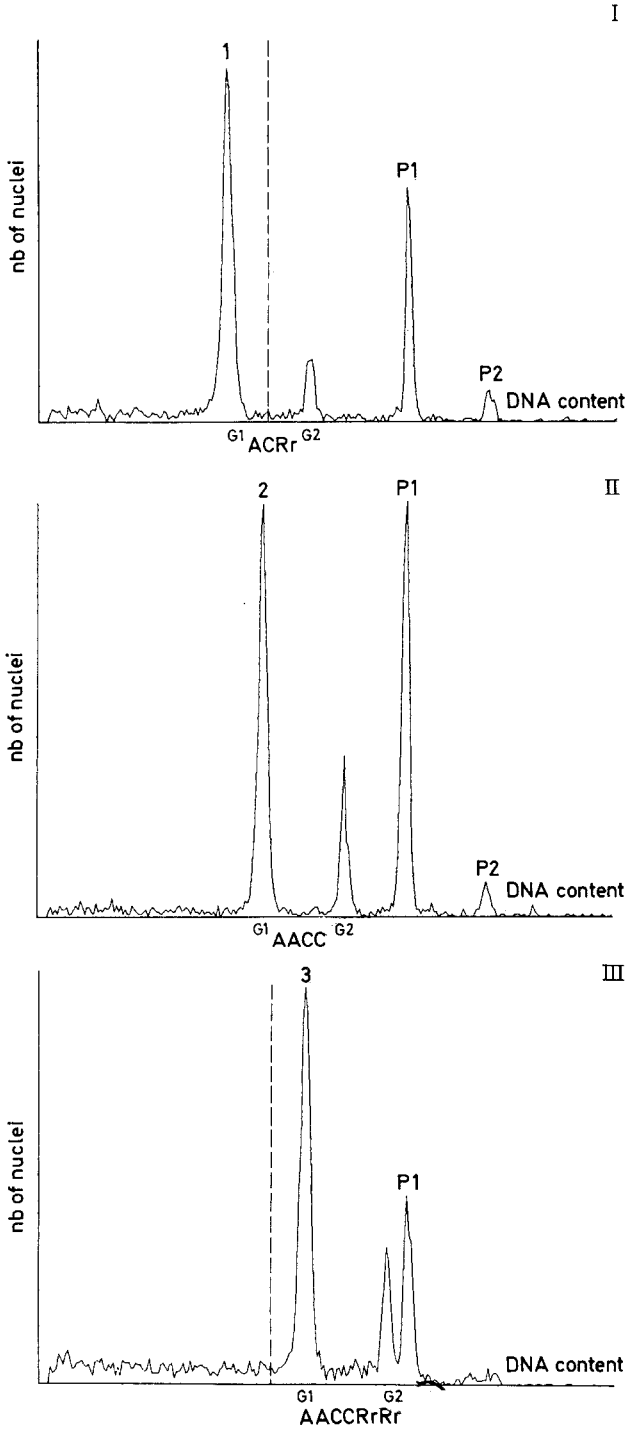

Fig. 2 Histograms used to select hybrids from rapeseed seedlings. A pea leaf fragment is used in each case as an internal standard, with its G1 and G2 peaks indicated by *P1* and *P2*. The central diagram (**II**) shows histograms obtained with rapeseed carrying 38 chromosomes, with peak no. 2 as G1. Triploid hybrids ($2n = 28$) on diagram **I** can be identified by a backward shift of their G1 peak (peak 1) with respect to that of pea. Similarly, amphidiploid hybrids ($2n = 56$) on diagram **III** show a G1 (peak 3) shifting towards P1

unexpected situations such as the finding of rapeseed plants derived from the small-seed population.

Flow cytometry was therefore used in subsequent experiments to quantify more precisely the production of interspecific hybrids. Larger samples of small seeds were assessed by flow cytometry in order to determine the proportion of triploid hybrids in the progeny of each genotype. Table 2 summarizes the results obtained for each line and hybrid. All of the genotypes showed more than 90% interspecific hybrid seeds in their progeny except for 'Dakkar' × 'WT5' (due to the occurrence of rapeseed seeds) and 'Miyuki' (because of the occurrence of rapeseed and a significant proportion of trigenomic amphidiploid plants). Other genotypes showed lower proportions of amphidiploids, but on the whole eight out of the ten did give rise to some AACCRrRr plants.

On the basis of the harvest data and the determination of genomic constitution, it was possible to evaluate more precisely the number of interspecific hybrid seeds per 100 pollinated flowers. This number was quite variable depending on the cross (Table 3) and that was confirmed by a factorial experiment. Graphical representations as well as mathematical tests showed that the data fit the normality prerequisite of the analysis of variance (data not shown). The model was highly significant with 96% of the variability explained (Table 4). Resolution of variance into its basic components showed that there was a highly significant interaction between factors nos. 1 (original background) and 2 (hybrid state), showing that the effect of the genetic background on the hybridization potential depended on whether lines or hybrids are considered.

The multiple classification of means was therefore performed separately for lines on one side and hybrids on the other. Student Newman and Keuls groupings for means per level of factor no. 2 are given on Fig. 3, together with an interaction graphic. Except for 'Drakkar' taken as a line, the spring genotypes were separated in both cases from the winter types. It looks as if 'Drakkar' was the genetic background that mostly gave rise to the interaction because the response was lowered when combined to 'Westar T5'. The four other hybrids showed higher means than the corresponding line.

Observations on F_1 interspecific hybrids

Smaller seeds displayed germination rates ranging between 51% and 65% depending on the genotype.

Herbicide spraying of large-seed-derived plants led to the expected 1:1 (resistant/susceptible) ratio (data not shown). Segregation results for Basta resistance among triploid and amphidiploid hybrids are listed on Table 5. Two genotypes out of the five exhibited an excess of resistant plants in their progenies.

Pollen fertility of the flowering triploid hybrids was on the whole very low: most of the plants were sterile (class 1), and only a few of them belonged to classes 2 and 3 (Table 6). Of the plants belonging to class 1 21% produced at least 1 seed; for classes 2 and 3 the percentages were 26%and 31%, respectively, with most of the plants within these latter two classes not giving any seed at all. Moreover, the contribution to the total seed set of

Table 2 Percentages of plants according to their genomic constitution in plants derived from small seeds in each parental genotype

Cross	Number of plants	Genome structure, chromosome number			
		ACRr,28	AACCRrRr,56	AACC,38	AC,19
Bru × RrRr	253	92.89	1.19	5.93	0.00
Dra × RrRr	248	92.34	0.00	7.66	0.00
Miy × RrRr	238	75.21	12.61	11.34	0.84
Sam × RrRr	251	97.21	1.20	1.20	0.40
Hob × RrRr	212	99.53	0.00	0.47	0.00
{Bru × WT5} × RrRr	608	92.76	0.33	6.41	0.49
{Dra × WT5} × RrRr	777	72.20	0.26	27.54	0.00
{Miy × WT5} × RrRr	377	93.10	1.06	5.84	0.00
{Sam × WT5} × RrRr	438	97.49	1.14	1.37	0.00
{Hob × WT5} × RrRr	384	98.44	0.26	0.78	0.52

Table 3 Harvest data according to the parental genotype

Cross	Pods/ 100 flowers	Seeds < 1.6 mm/pod	Hybrid seeds/ 100 flowers	Hybrid seeds/ plant
Bru × RrRr	56.3	0.05	2.7	8.3
Dra × RrRr	73.6	0.40	27.6	210.6
Miy × RrRr	14.2	0.12	1.4	5.3
Sam × RrRr	75.4	0.35	26.4	206.1
Hob × RrRr	54.6	0.76	41.3	202.1
{Bru × WT5} × RrRr	71.7	0.56	37.4	175.0
{Dra × WT5} × RrRr	73.7	0.40	15.1	89.8
{Miy × WT5} × RrRr	58.1	0.63	28.9	393.6
{Sam × WT5} × RrRr	86.9	1.03	88.6	445.0
{Hob × WT5} × RrRr	73.7	0.94	68.4	556.5

Table 4 Analysis of variance table for the crossed effects of genetic background (Genback) and hybrid state (T5) factors. Genback and T5 effects, respectively, are tested towards the interactions Genback × Blocks and T5 × Blocks

Source	*df*	Mean square	Pr > F
Model	33	1,057.8	**0.0001**
Error	15	132.6	
Corrected total	48[a]		
$R^2 = 0.96$			

Source	*df*	Mean square	Pr > F
Genback	4	3,825.2	0.0001
T5	1	10,595.8	0.0013
Blocks	4	214.4	0.2215
Genback × T5	4	1,789.6	**0.0001**
Genback × Blocks	16	68.9	0.8972
T5 × Blocks	4	161.1	0.3454

[a] One missing value has been evaluated

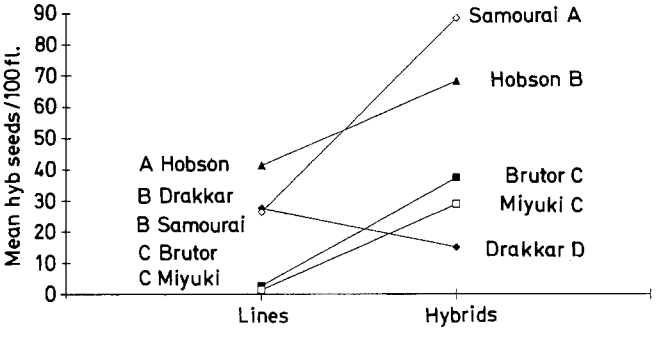

Fig. 3 Interaction diagram according to the levels of the second factor (hybrid state). Means with the same letter are not significantly different at the 5% level. Student Newman and Keuls groupings for lines and hybrids are independent

classes 1, 2 and 3 were 96%, 3% and 0.95%, respectively. The number of harvested seeds per plant was also higher in class 1 (3.12) than in classes 2 (1.25) and 3 (1.02). Consequently, no correlation was recorded between male fertility and seed set in the following generation (Table 7).

Discussion

We confirmed that the 'Westar T5' line is a single-copy transformant homozygous for the *bar* gene. The integrity of the left border has been checked by specific polymerase chain reaction (PCR) primers, and it has been shown that the transgenic line carries no sequence of plasmid origin (C. Opsamer, personal communication). A precise molecular description of a transformant

Table 5 Basta resistant (R) and susceptible (S) triploid and amphidiploid hybrids according to the maternal genotype

Cross	R	S	χ^2[a]
{Bru × WT5} × RrRr	163	151	0.38
{Dra × WT5} × RrRr	114	95	1.55
{Miy × WT5} × RrRr	184	141	5.43*
{Sam × WT5} × RrRr	215	176	3.69
{Hob × WT5} × RrRr	203	154	6.45*
Total	879	717	16.24**

[a] Yates-adjusted Chi-square. Distributions differing from the 1:1 theoretical ratio are indicated by * (5% level) or ** (1% level)

Table 6 Percentages of totally sterile (class 1) and partially fertile (0–10% for class 2; 10–30% for class 3) triploid interspecific hybrids according to the maternal genotype

Cross	Number of plants	Male fertility classes		
		1	2	3
Bru × RrRr	204	94.3	3.1	2.6
Dra × RrRr	200	95.7	3.8	0.5
Miy × RrRr	85	88.9	11.1	0.0
Sam × RrRr	191	93.9	3.4	2.7
Hob × RrRr	187	91.9	8.1	0.0
{Bru × WT5} × RrRr	376	88.9	8.8	2.3
{Dra × WT5} × RrRr	351	90.3	7.6	2.1
{Miy × WT5} × RrRr	224	86.6	7.5	5.9
{Sam × WT5} × RrRr	287	86.0	8.9	5.1
{Hob × WT5} × RrRr	248	87.6	9.3	3.1
Total	2,353	90.1	7.1	2.7

is an absolute prerequisite to its commercialization as a variety.

Homozygosity has also been checked by a testcross analysis to a susceptible line of the same cultivar. Other segregating populations in which this line was involved as a parent combined to susceptible varieties, such as doubled-haploid lines that showed the expected 1:1 ratio (data not shown), confirmed this data. Finally, all of the rapeseed hybrid mother plants prepared for the field trial were resistant. There has been no silencing of the transgene in a heterozygous form.

Detection of the interspecific hybrids was mostly based on cytogenetics, as previously recommended by Eber et al. (1994). Our study showed that flow cytometry can advantageously replace tedious mitotic counts if the genomic constitutions of the hybrids (triploid and amphidiploid) and parents are different enough in terms of DNA contents. The combined use of seed sifting and flow cytometry allowed precise characterization of the seeds produced.

Although the maternal plants were male-sterile and the trial isolated 500 m from any other rapeseed field, significant amounts of large seeds showing the typical rapeseed genomic constitution were harvested from all of the maternal genotypes. Isoenzymatic studies demonstrated that some of these seeds originated from oilseed rape pollen contamination, which could explain their very irregular occurrence, and others from matromorphism (data not shown). A 500-m-wide isolation buffer was probably not enough to prevent pollinators from distributing foreign pollen.

The occurrence of some rapeseed plants from small seeds is probably due to shrivelling, the phenomenon being most visible in 'Drakkar' × 'WT5' progeny. On the other hand, the spontaneous occurrence of rapeseed haploids has been previously described by Renard and Dosba (1980), but might also be favored by interspecific hybridization (Prakash and Hinata 1980).

The most frequent genomic constitution in small seeds was the expected ACRr with 28 chromosomes, thereby showing that interspecific barriers were largely overcome. Previous reports based on restriction fragment length polymorphisms (RFLP) had shown that *R. sativus* (Song et al. 1988) as well as *R. raphanistrum* (Warwick and Black 1991) belong to the same lineage as *B. rapa* and *B. oleracea*. The relative proximity of the Rr genome with the A and C genomes might therefore explain the wide hybridization with *B. napus*.

Possible introgression has been closely linked to meiosis in the hybrids. Triploid hybrids are much more likely than the related trigenomic amphidiploids to give rise to recombination between the A or C and Rr genomes because of the high level of chromosome pairing in their meiosis compared to that of the corresponding AC rapeseed haploid (Kerlan et al. 1993; Eber et al. 1994). Amphidiploids are of common occurrence in *Brassicaceae* (U 1935). While one genotype, i.e. 'Miyuki', showed a clear tendency to produce such AACCRrRr individuals, the phenomenon seemed to occur in most genotypes, albeit generally at a low level. These amphidiploids could result either from the production of unreduced gametes or from endomitosis following fertilization. This last hypothesis is supported by the occur-

Table 7 Seed set on the BC_1 production trial cumulated on all of the genotypes according to the male fertility class

Male fertility classes	Number of plants	Percentage of plants with at least 1 seed[a]	Number of seeds	Percentage of total seed set
1	1,710	21.11 A	5,335	96.01
2	135	25.93 A	169	3.04
3	52	30.77 A	53	0.95

[a] Percentages with the same letter are not significantly different at the 5% level

rence of Basta susceptible amphidiploids derived from Basta resistant rapeseed hybrids 'Miyuki' × 'Westar T5' and 'Brutor' × 'Westar T5' (data not shown). It is likely that the ability of these amphidiploids to produce a progeny is higher than that of triploid hybrids because of the regularity of their meiosis. Preliminary data have shown that a high chromosome number is maintained in the progeny of oilseed rape × kale amphidiploid hybrids obtained spontaneously by selfing or backcrossing (Chèvre et al. 1994).

The oilseed rape genotypes were chosen to represent a large diversity of *B. napus* cultivars: spring and winter types, forage and oleaginous, European and Asiatic varieties combined with the Canadian 'Westar' cultivar. On the other hand, the wild radish population used has been shown to be highly polymorphic with an intrapopulation diversity that contributes up to 72% of the total diversity (data not shown).

Our results demonstrate that it is fairly easy to produce transgenic interspecific hybrids under natural conditions by largely reducing interspecific barriers using a male-sterile recipient and high pollen pressure from the wild species. However, different rapeseed genotypes vary greatly in their ability to produce interspecific hybrids under such conditions. Although the breakdown of the variation has not been total, there were clearcut differences between spring types, except for 'Dakkar', and winter types. The hybrid state also seemed to increase this potential, except for 'Dakkar', which gave rise to the interaction between the two factors due to a lowering of the response. The classification obtained is close to the one observed for fully fertile varieties for yield, i.e. spring types < winter types and lines < hybrids. In order to confirm these results and to sort possible environmental or year effects, we have setup the same trial for a second-year experiment.

Although Basta resistance did not follow the expected 1 : 1 segregation ratio in two out of five progenies, it seems that this shifting towards resistance was mostly due to failures in detecting susceptible plants when using the non-destructive test. Resistance tests in he BC_1 progeny will be helpful in confirming this hypothesis. Nevertheless, we have shown that Basta resistance is expressed in interspecific backgrounds and therefore might be transmitted to the wild, assuming that subsequent backcross progenies are generated.

Production of following generations will be very much dependent on fertility. Although male fertility of the triploid hybrids was on the whole very low, a singificant number of "BC_1" seeds were harvested, showing that seed set depends on female fertility. Indeed, plants belonging to fertility classes 2 and 3 did not contribute much to the seed set. Interspecific hybrids were produced on a male-sterility-inducing cytoplasm, but it was difficult to distinguish between sterility linked to interspecific hybridization and the maintenance of cytoplasmic male sterility. While it appears as if there has been in some cases a partial restoration of fertility by *R. raphanistrum*, hybrids obtained from fully fertile parent with the use of embryo rescue also show low fertility (Kerlan et al. 1992).

Various genotypes may also contribute differently to the hybridization in subsequent generations. We have therefore set up a similar completely randomized trial aimed at the production of BC_1 interspecific hybrids. Work is in progress to define the genomic constitution of the plants obtained from this trial. Previous data have shown that most of the "BC_1" plants from ACRr triploid hybrids (Eber et al. 1994) carried either the expected ACRrRr constitution (37 chromosomes) or were AACCRrRr amphidiploids (56 chromosomes) similar to the ones obtained in F_1 crosses (Chèvre et al. 1994). Two evolution processes can therefore be predicted: (1) the return to a diploid wild species through the lowering of chromosome number in the subsequent backcrosses; the transgene dispersal would then be conditioned by recombination between the A or C and Rr genomes, and (2) the possible maintenance of high ploidy levels, and particularly of amphidiploids as a new weedy species; the transgene dispersal would then be dependent upon the evolution and competitiveness of this new species.

Our study was restricted to the use of *Brassica napus* as the female as no male sterility is available in *R. raphanistrum*. The reciprocal cross might occur through the expression of self-incompatibility in *Raphanus*, as observed for crosses with *B. campestris* (Jorgensen and Andersen 1994). Up to now, there has been no evidence for the production of interspecific hybrids involving fully fertile *Brassica napus* under natural conditions, except for *B. campestris* and *B. juncea* (Scheffler and Dale 1994).

Our model of hybrid production used male sterility and high pollen pressure from the wild species. The main difference in rapeseed F_1 hybrid seed production fields or varietal association (such as hybrid and line composites) fields is the availability of pollen from rapeseed in large quantities. Under these conditions, interspecific crosses must occur in considerably lower amounts because of pollen competition, except if the male and female flowering periods in the rapeseed mother lines do not match well. Still under these conditions, we have shown that the influence of the parental genotype is critical in the hybridization rate. Even if interspecific hybridization frequencies are low under commercial practices, small seeds will most probably remain in the field and germinate either the year after or some years later. Maintenance of the triploid hybrids as well as amphidiploids will then depend very much on selection pressure, in our case on herbicide spraying. If the same herbicide is to be used the next year, these seeds will have an advantage. It therefore seems to be important to develop rational protocols and management strategies in and outside the field if such transgenic plants are to be released under commercial conditions in the near future. Our experimental data together with computerized modeling could be useful in the setting up of such protocols.

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