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Spontaneous hybridization between a male-sterile oilseed rape and two weeds

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Abstract Spontaneous interspecific hybrids were produced under natural conditions (pollination by wind and bees) between a male-sterile cybrid *Brassica napus* (AACC, 2n = 38) and two weeds *Brassica adpressa* (AdAd, 2n = 14) and *Raphanus raphanistrum* (RrRr, 2n = 18). After characterization by chromosome counts and isozyme analyses, we observed 512 and 3 734 interspecific seeds per m^2 for the *B. napus–B. adpressa* and *B. napus–R. raphanistrum* trials respectively. Most of the hybrids studied had the expected triploid structure (ACX). In order to quantify the frequency of allosyndesis between the genomes involved in the hybrids, their meiotic behavior was compared to a haploid of *B. napus* (AC). For the *B. napus–B. adpressa* hybrids, we concluded that probably no allosyndesis occurred between the two parental genomes, and that genetic factors regulating homoeologous chromosome pairing were carried by the *B. adpressa* genome. For the *B. napus–R. raphanistrum* hybrids, high chromosome pairing and the presence of multivalents (in 9.16% of the pollen mother cells) indicate that recombination is possible between chromosomes of different genomes. Pollen fertility of the hybrids ranged from 0 to 30%. Blackleg inoculation tests were performed on the three parental species and on the interspecific hybrids. BC_1 production with the weeds and with rapeseed was attempted. Results are discussed in regard to the risk assessment of transgenic rapeseed cultivation, F_1 hybrid rapeseed variety production, and rapeseed improvement.

Key words *B. napus* · *Brassicacae* weeds · Spontaneous interspecific hybridization · Cytogenetics · Blackleg resistance

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Introduction

The tribe *Brassicaceae* includes numerous species some of which, such as *Brassica napus* (rapeseed), are important crops. A considerable number of interspecific hybridization studies have been performed with rapeseed mostly for the determination of phylogenetic relationships (Mizushima 1980) or for breeding purposes. Although some crosses involving several diploid species are successful without special aid, embryo rescue is often necessary. Most of the interspecific hybrids involving *B. napus*, an allotetraploid, have been produced only by using in-vitro methods (Prakash and Hinata 1980; Namai 1987; Glimelius et al. 1991).

Hybrids of *B. napus–B. adpressa* were reported by Harberd and McArthur (1980) and Kerlan et al. (1992) using in-vitro ovary culture. Heyn (1976) has used *Raphanobrassica* (RRCC, 2n = 36), to incorporate characters from *Raphanus sativus* L. (RR, 2n = 18) into rapeseed (*B. napus*, AACC, 2n = 38). *Raphanobrassicas* were obtained by sexual crosses between *R. sativus* and *B. oleracea* or *B. campestris*, without the need for embryo culture (Karpechenko 1924; Chopinet 1944; McNaughton 1973; Mizushima 1980). Hybrids between *B. napus* and *R. sativus* (Takeshita et al. 1980) and *B. napus* and *R. raphanistrum* L. (RrRr, 2n = 18) (Kerlan et al. 1992) were obtained using embryo rescue.

In order to assess the extent of outcrossing of rapeseed to weedy relatives in cultivated areas and the purity of F_1 rapeseed varieties produced by using male sterility, we attempted spontaneous hybridization under natural conditions between a male-sterile rapeseed and two weeds: *B. adpressa* Boiss. (Moench) (*Hirshfeldia incana* L. Lagrèze-Fossat, AdAd, 2n = 14), a mediterranean species, and *R. raphanistrum* L. (RrRr, 2n = 18), a European species. This would also allow rapeseed improvement by introgression of genes controlling agronomic characters (such as disease resistance).

In this paper, production of F_1 interspecific hybrids, between a male-sterile rapeseed and two weeds is de-

scribed. Characterization of the plants obtained was performed by cytological and agronomical studies. Their ability to produce progeny is discussed.

Materials and methods

Material

The female was a male-sterile cybrid of a Spring rapeseed line (Fu Brutor) described by Pelletier et al. (1983). The populations of *B. adpressa* and *R. raphanistrum* were locally collected.

Field experiments were set up in two different trials isolated from any other rapeseed by 500 meters. The rapeseed female parent and the wild species were arranged in plots consisting of five alternating rows 2 m long. One of the trials, involving *B. adpressa* (Fu Bad trial), had 25 replications (250 m^2). The second one, involving *R. raphanistrum* (Fu Rr trial), had six replications (60 m^2). For the female parent, the number of flowers, pods and seeds per m^2 was assessed. The germination ability of 100 seeds collected was determined in Petri dishes (25 °C).

To assess the seed production of each interspecific hybrid combination, the seeds were sown in two designs. The first one was in an isolated field of interspecific hybrids (20 m^2) surrounded by their corresponding parental weed population, the second one was sown in a Spring rapeseed field consisting of two rows 1.5 m long of each F_1 interspecific hybrid.

To compare the results of the interspecific cross *B. napus* × *R. raphanistrum* with the published results for *B. napus*–*R. sativus* hybrids (Takeshita et al. 1980), crosses were performed between the two *Raphanus* species. The wild population of *R. raphanistrum*, locally collected, and *R. sativus* var. Pegletta were used for this study.

To evaluate the frequency of allosyndesis between the genomes, the meiotic behavior of haploid plants of Brutor was used as a comparison. Brutor haploid plants were generated using microspore in-vitro culture and kindly provided by M.O Lucas (INRA, Le Rheu).

Cytological methods

Chromosome counts were made on root meristems from germinated seeds. Material was treated with a solution of 8-hydroxyquinoline (0.29 g/l) for 4 h at room temperature, then fixed with acetic acid-ethanol (1:3) for at least 18 h at 4 °C. Meristems were hydrolysed in 1M HCl for 10 min at 60 °C, then stained with Schiff's reagent. For the meiotic studies, floral buds were fixed in Carnoy's solution (ethanol–chloroform–acetic acid, 6:3:1) for 24 h and stored in 50% ethanol. For the observation of pollen mother cells (PMCs) at the MI stage, anthers were squashed and stained in a drop of 1% acetocarmine solution. Mature pollen was coloured with acetocarmine, and pollen fertility was estimated by the percentage of fully-stained grains. Counts were made on 200–1 000 grains from three flowers. From the field and the cytological observations, a 3-class scale was adopted, the first one corresponding to 0% of stained pollen grains, the second ranging from 1 to 10%, the third from 11 to 30%.

Isozyme analysis

Crude extracts of leaves were prepared by crushing the tissue in extraction buffer containing tris HCl 0.1 M pH 7.5 and 1% w/v of glutathione. Gel/electrode buffers for starch gels were histidine/tris citrate pH 7.0 for phosphoglucomutase (PGM) and phosphoglucoisomerase (PGI) analyses. Gel/electrode buffers were morpholine citrate pH 6.1 for leucine aminopeptidase (LAP) and 6-phosphogluconate-dehydrogenase (6-PGD) analyses. These buffers were described by Shield et al. (1983). The staining protocol for LAP is given by Arus and Orton (1983). The three enzymes 6-PGD, PGM and PGI were stained as specified by Vallejos (1983).

Blackleg resistance test

The method of Williams and Delwiche (1979) was used for screening the seedlings for resistance to blackleg, *Leptosphaeria maculans* (Desm.) Ces. et de Not. (*Phoma lingam* (Tode) Desm.). Each half cotyledon of 5-day-old seedlings was wounded with a needle, inoculated with a pycnidiospore suspension of an isolate (10^7 spores/ml) and incubated at 21 °C. Symptom development evaluation was done 14, 18 and 21 days after inoculation using a 9-class scale based on lesion size. Only plants of class 1, with no enlargement of the inoculation lesion, were considered as resistant. The other classes were determined by the size of blackening tissue, and plants showing medium to large lesions corresponding to symptoms of class 2, 3 or 4 were considered as moderately-resistant. Classes 5 to 8 including very large lesions, and class 9 corresponding to collapsed cotyledons, were considered as susceptible.

Results

Seed production

In order to have an overlapping flowering period with *B. adpressa* and *B. napus* under our conditions, *B. adpressa* was sown 2 weeks before rapeseed and *R. raphanistrum*, and the three species flowered at the same time. Pods formed on the rapeseed parent were small and deformed, containing two seed-size groups, the first one with seeds bigger than 2 mm in diameter, the second one with seeds of approximately 1.6 mm in diameter. Six-hundred seeds from the Fu Bad trial and 7 018 seeds from the Fu Rr trial were collected per m^2, 14.6% of the seeds collected from the first trial were of the larger size group, as were 46.8% in the second trial. Since the large seeds were not hybrids (see below), Table 1 shows only the results based on the smallest seed-size group. As a reference, Fu Brutor seeds were 2 mm diameter approximately, while *B. adpressa* and *R. raphanistrum* were 1 to 1.2 mm and 2 to 2.5 mm diameter, respectively.

Germination percentages varied from 92.1 to 59.3 for seeds harvested from the Fu Bad and Fu Rr trials respectively.

Fu Brutor – *B. adpressa* trial

Isozyme markers. Fu Brutor and *B. adpressa* were compared for the allelic mobility at ten loci revealed using four isozyme systems: PGI, PGM, 6-PGD and LAP. The allozymes of six loci (Pgi 2, Pgm 3, 6-Pgd 1, 6-Pgd 2, 6-Pgd 2′ and Lap 1) of *B. adpressa* always had different mobilities from those of the rapeseed variety used, re-

Table 1 Seed production in the female parent Fu Brutor (diameter ≤ 1.6 mm)

Trials	Seed number per m^2	Seed number per pod	Pod number per 100 flowers	% Germination
Fu Bad	512	1.15	1.67	92.1
Fu Rr	3734	0.32	29.80	59.3

gardless of the variability of the wild species. The two types of seeds harvested in the trial were analysed. All the large seeds (diameter > 1.6 mm) were found to be *B. napus*; all the small seeds (diameter ≤ 1.6 mm diameter) were interspecific hybrids and presented bands of both parental species.

Cytological observations

Chromosome number. All the plants obtained from larger seeds had 38 chromosomes; the chromosome numbers of plants developing from smaller seeds are shown in Table 2. Of the 85 plants studied, one had 19 chromosomes probably corresponding to a haploid plant of *B. napus* with an AC genomic constitution. The 84 others had the expected chromosome number (2n = 26) for the ACAd triploid constitution.

Chromosome pairing. The meiotic behavior of eight plants is shown in Table 3. The rest of the plants displayed a very low number of PMCs because of an early degeneration prior to meiosis. Two groups of plants could be distinguished, the first one with 25.6% and the second one with 50.0% chromosome pairing, respectively. These two groups were characterized by two different chromosome pairing distributions (Fig. 1a). In group I, the number of univalents ranged from 18 to 22, whereas in group II this value ranged from 8 to 18 with no multivalents observed. Assuming that the chromosomes of *B. adpressa* were not involved in the bivalents observed, the percentage of chromosomes paired on the 19 remaining chromosomes (AC) would be 35.1% for group I and 68.5% for group II.

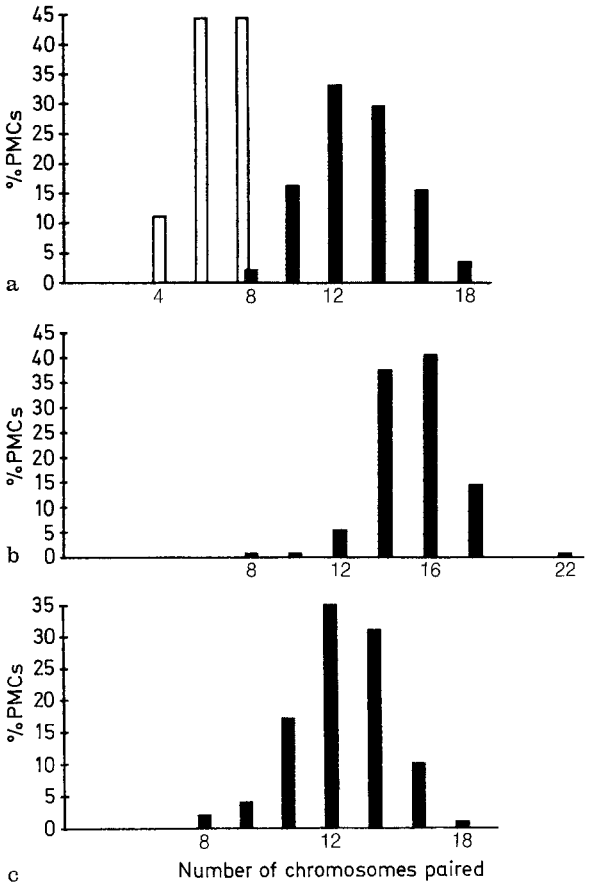

Fig. 1a–c Distribution of paired chromosomes in the interspecific hybrids and haploid plants of the parental *B. napus* line. **a** Fu Bad hybrids (26 chromosomes) with group-I in *white* and group-II in *black*. **b** Fu Rr hybrids (28 chromosomes). **c** Haploid plants of Brutor (19 chromosomes). PMCs, pollen mother cells

Table 2 Cytological characterization of the seeds (diameter ≤ 1.6 mm) harvested on the *B. napus* female parent, with the likely genomic constitution in parenthesis

Trials	Number of plants studied	Number of chromosomes			
		19	26	28	56
Fu Bad	85	1 (AC)	84 (ACAd)		
Fu Rr	189			188 (ACRr)	1 (AACC RrRr)

Table 3 Meiotic behavior of the interspecific hybrids and of the haploid plants of Brutor, and their distribution in the three classes of pollen fertility (*I* Univalent, *II* bivalent, *III* trivalent, *IV* quadrivalent (range in parenthesis) Fertility classes: *1* 0% of stained pollen grain, *2* 1–10%, *3* 11–30%)

Plants	Number of plants	Number of PMCs observed	2n	Meiotic behavior				% Chromosomes paired	Number of plants per fertility class		
				I	II	III	IV		1	2	3
Fu Bad group I	2	36	26	19.33 (*18–22*)	3.34 (*2–4*)			25.64	2		
Fu Bad group II	6	142	26	12.98 (*8–18*)	6.51 (*4–9*)			50.05	3	2	1
Fu Rr	7	131	28	12.72 (*6–20*)	7.47 (*4–9*)	0.01 (*0–1*)	0.08 (*0–1*)	54.58	2	3	2
Brutor haploid	2	100	19	6.44 (*1–13*)	5.99 (*3–9*)	0.10 (*0–1*)	0.07 (*0–1*)	66.10			

In three plants, 10–14% of the cells had chromosome numbers ranging from 38 to 60.

After the MI stage, unbalanced divisions and monads, dyads, triads, or abnormal tetrads were observed.

Pollen fertility. The percentage of acetocarmine-stained pollen grains ranged from 0 to 30% (Table 4). In 54 plants studied, 87.0% were sterile (class 1) and 13.0% poorly fertile (classes 2 and 3). Pollen fertility was also assessed for the plants observed in the meiotic studies (Table 3). The two plants of the low-pairing group I were sterile, whereas the plants belonging to group II were represented in all three fertility classes.

Blackleg resistance test. Compared with the plants of Brutor, which were all susceptible, the 21 inoculated plants of *B. adpressa* were resistant, not showing any symptom evolution of the initial lesion (Table 5). Of the 28 interspecific hybrid plants, 46.4% were in class 1 and the remaining 53.6% were susceptible. No intermediate resistance was observed in the hybrids (classes 2 to 4).

Seed production of Fu Bad hybrids. The interspecific hybrids were vigorous and had a morphology intermediate between the two parents. The few pods that developed on the hybrids were as small as those of the *B. adpressa* parent. In the isolated area where the F_1 hybrids were surrounded by *B. adpressa*, 158 hybrid plants were observed and 18.3% carried small pods (1 925 pods were collected). The 65 seeds obtained were very small (< 1.2 mm diameter). The number of pods per 100 flowers was 7.5 and the number of seeds per m^2 was 3.2.

In the rapeseed field, ten plants were studied, seven carried pods and 170 seeds were collected (from 1.2 to 1.6 mm diameter) (Table 6).

Table 4 Pollen fertility of the interspecific hybrids obtained according to three classes

Trials	Number of plants studied	Fertility classes		
		1	2	3
Fu Bad	54	47	4	3
Fu Rr	101	66	23	12

Table 5 Blackleg-resistance test of the parental species and of the interspecific hybrids. Evaluation of symptom development was done according to a 9-class scale, from resistant to susceptible plants

Plant	Number of plants tested	Susceptibility classes				
		1	3	5	7	9
Fu Brutor	28	0	0	0	4	24
B. adpressa	21	21	0	0	0	0
R. raphanistrum	42	18	0	3	0	21
Fu Bad	28	13	0	2	2	11
Fu Rr	153	19	0	10	14	110

Fu Brutor – *R. raphanistrum* trial

Isozyme markers. The two parental species were compared for four isozyme systems: PGI, PGM, 6-PGD and LAP. A preliminary study revealed that the rapeseed variety used did not segregate, behaving like a pure line, whereas the *R. raphanistrum* population was highly variable. One of the four allozymes described at the Pgi 2 locus of *R. raphanistrum* had the same mobility as one of the rapeseed allozymes. In order to avoid this ambiguity, only four of the nine loci studied were used for distinguishing the two species: Pgm1, 6-Pgd1′, 6-Pgd2′ and Lap1. Seedlings obtained from the two seed sizes were analysed. The larger seed class corresponded to *B. napus*. The smaller seed class (diameter $\leq$ 1.6 mm) produced seedlings with bands of both parental species as expected for interspecific hybrids. All the following studies were performed on seedlings developed from the small seeds.

Cytological observations

Chromosome number. Among the 189 plants observed, 188 were triploid (2n = 28) corresponding to the expected ACRr genomic constitution, and one had 56 chromosomes probably corresponding to the amphiploid AACCRrRr constitution (Table 2).

Chromosome pairing. The seven hybrid plants studied displayed from four to nine bivalents per PMC, with a percentage of chromosome pairing of 54.58% (Table 3, Fig. 1b). Trivalents and quadrivalents were observed in 9.2% of the PMCs. Misdivisions were often observed after MI with the formation of dyads.

Table 6 Seed production in F_1 interspecific hybrids with their two recurrent parents as male

Interspecific F_1 hybrids	Male species	Number of plants observed	% Plants with pods	Seed number per pod	Seed number per plant observed
Fu Bad	*B. adpressa*	158	18.35	0.03	0.41
	B. napus	10	70.00	[a]	17.00
Fu Rr	*R. raphanistrum*	281	8.54	0.09	0.07
	B. napus	20	85.00	0.24	28.35

[a] Missing data

Pollen fertility. According to the 3-class fertility scale, of the 101 plants observed, 65.4% were sterile and 34.6% presented 1–30% of stainable pollen (Table 4). The plants sampled for the meiotic studies belonged to different classes (Table 3). Stained pollen grains varied in size.

Blackleg resistance test. Compared with the susceptible pure line Brutor, of the 42 plants of *R. raphanistrum* studied, 42.9% were resistant (class 1) and the rest susceptible (classes 5–9). For the interspecific hybrid plants, 12.4% were resistant and the remaining plants susceptible, classes 5–9 (Table 5). No plants with intermediate symptoms (classes 2–4) were observed.

Seed production of Fu Rr hybrids. The interspecific hybrid plants were as vigorous as oilseed rape and had an intermediate phenotype for most parental traits except for flower colour (bright yellow flowers of Brutor) and pod development (small deformed pods). In the isolated field where the interspecific plants were surrounded with *R. raphanistrum*, 8.5% of the 281 plants observed set pods. From the 234 pods harvested, the 20 seeds obtained were round and larger than the F_1 seeds (> 1.8 mm diameter). The number of pods per 100 flowers was 0.5 and the number of seeds per m^2 was 1.0. In the rapeseed field 20 plants were studied, 17 carried pods, and 567 seeds were collected (> 1.6 mm diameter) (Table 6).

Complementary studies

The results previously reported between *B. napus* and *Raphanus* concerned only *R. sativus* (Takeshita et al. 1980). In order to compare the hybrids produced in our study with the *B. napus–R. sativus* hybrids, crosses between *R. sativus* and *R. raphanistrum* were performed in the greenhouse. No in-vitro culture was needed to obtain seeds.

A PG1 system was used to choose the parents with distinguishable allozymes. Seeds obtained from the crosses were sown in the greenhouse and the plantlets analysed. They presented both parental bands and were characterized as interspecific hybrids.

The meiotic behavior of the two parents was established and showed the expected nine bivalents. In the 120 PMCs of the six F_1 hybrids examined, 95.79% of the chromosomes were paired. Different configurations were observed: 1 I + 7 II + 1 III in 75.8% of the PMCs, 9 II in 15.8%, 7 II + 1 IV in 7.5% and 6 II + 2 III in 0.8%.

The two *Raphanus* parents had 90% of stained pollen grains. For the six interspecific hybrids observed the percentage of stained pollen grains ranged from 56.6% to 66.3%.

Fifty PMCs of each of two haploid plants of Brutor were studied (Table 3). Bivalents ranged from 3 to 9 and multivalents occurred in 17% of the PMCs. The percentage of chromosomes paired was 66.10% (Fig. 1c).

Discussion

To our knowledge, this is the first documented report of hybrid seed production by natural pollination between a male-sterile rapeseed and two weeds, *B. adpressa* and *R. raphanistrum*. The hybrids were confirmed by chromosome counts, which allowed the genomic structure of the seedlings observed to be determined, and by specific zymograms for several isozyme loci. This characterization also revealed the presence of non-hybrid matromorphic seeds which may have arisen by apogamy from a restitutional 1n egg cell. This may occur in cases of low compatibility and was previously described by Heyn (1977). However, it is also possible that they were the result of pollen contamination by the same species because of the presence of numerous pollinator insects and an inadequate isolation distance (500 m).

More seeds per m^2 were obtained in the Fu Rr trial than in the Fu Bad trial. These results are in agreement with the taxonomic relationships of these species as established by Song et al. (1988) using nuclear DNA analyses and by Warwick and Black (1991) with chloroplast DNA, and showed that *Raphanus* species were more closely related to *B. napus* than *B. adpressa*. However, higher variability in the wild *Raphanus* population compared to that observed in *B. adpressa* (Kerlan et al. 1992) might also explain our results. On the other hand, we cannot exclude the possibility that the radish origin of the male-sterile cytoplasm improved the crossability.

The percentage of germination of the Fu Bad hybrid seeds was the same as that of the two parents. However, the dormancy of the *R. raphanistrum* seeds (data not shown) seems to be expressed in the Fu Rr hybrids since the percentage of germination was lower than that observed for rapeseed seeds.

The majority of the plants observed had the expected triploid ACX structure. However, during mitotic analyses of the Fu Bad seedlings, a haploid plant of *B. napus* was detected. Such a phenomenon was previously described by several authors, after interspecific hybridizations (Prakash and Hinata 1980). Spontaneous haploids also occur in *B. napus*, but the frequency depends on the origin of the rapeseed genotype (Renard and Dosba 1980). Observations of cells in mitosis from Fu Rr seedlings revealed the presence of one plant with an AACCRrRr amphidiploid structure (56 chromosomes). This phenomenon, which is normally due to the production of unreduced gametes, is known to occur in the *Brassiceae* with a relatively high frequency (Heyn 1977).

Some difficulties arose in the observation of meiotic behavior of the Fu Bad hybrids, due to the early degeneration of PMCs prior to meiosis. This was previously described by Mizushima (1980) in *B. adpressa–B. campestris* interspecific hybrids (AAd, $2n = 17$). The ACAd hybrids we studied formed two groups; the first group (group I) with a low chromosome pairing mode of 6 and the second one (group II) with a mode of 12 (Fig. 1a). The latter had the same mode as the haploid of

Brutor, whereas group I had fewer paired chromosomes. This may be due to the existence of genetic factors preventing homoeologous chromosome pairing in the population of *B. adpressa* used. Such factors have been described in the *Triticinae* by Sears and Okamoto (1958) and Chen and Dvorak (1984). In the ACAd hybrids, no multivalents occurred and the number of paired chromosomes was not higher than in the haploid Brutor. Most likely there was no allosyndesis between the two parental genomes. Some cells presented higher chromosome numbers, possibly due to cytomixis which has already been observed in interspecific *Cruciferae* hybrids (data not shown). Such a phenomenon has been previously described in *Hordeum* by Kamra (1960) and in triploid hybrids of *Prunus* by Salesses (1970).

For the Fu Rr hybrids, only one group was established (Fig. 1b), showing more chromosomes paired than in the haploid rapeseed parent. The results from the Rr genome (*R. raphanistrum*) may be compared with those of the R genome (*R. sativus*). In fact, cytological studies of the interspecific hybrids *R. sativus–R. raphanistrum* revealed that, in 84.1% of the PMCs, one trivalent or one quadrivalent occurred and that pollen fertility of the two parents was reduced to approximately 34% in the hybrids. It would appear that the two species differ only by a reciprocal translocation. Partial homology between the R genome of *R. sativus* and the A and C genomes has been already reported (Mizushima 1980; Prakash and Hinata 1980; Namai 1987). This homoeology may allow recombination between the three genomes. This is supported by the fact that Pellan-Delourme and Renard (1988) introduced a fertility restorer gene from *R. sativus* into *B. napus*. Similarly, homoeology between the *B. napus* and *R. raphanistrum* genomes was confirmed by the high number of chromosomes paired and by the presence of multivalents which may allow recombination between chromosomes of the different genomes.

Whatever their meiotic behavior, the plants were present in three different classes of pollen fertility ranging from 0 to 30%. Therefore, at this low level of fertility, no relation could be established between chromosome pairing and pollen fertility. However, the presence of more plants with high fertility in the Fu Rr hybrids (34.6%) than in the Fu Bad hybrids (13.0%), may be explained by the *Raphanus* cytoplasm origin of the *B. napus* parent used (Pelletier et al. 1983), with the R genome carrying restorer genes.

The blackleg inoculation tests revealed two types of resistance for the two wild species. Although all the *B. adpressa* plants were resistant, only 46.4% of the FuBad hybrids were. If resistance was under the control of several genes, susceptible plants would not be observed in the few plants tested in the wild population whereas susceptibility would be revealed in the haploid genome present in the F_1 interspecific hybrid. On the other hand, the regulation of the resistance genes might be disrupted for some genotypes in the interspecific background.

The resistant plants of the *R. raphanistrum* population tested showed a hypersensitive reaction, as reported by Roy (1978) in *B. juncea* (AABB, $2n = 36$) and *B. carinata* (CCBB, $2n = 34$). The *R. raphanistrum* population segregated for this character, with 42.9% resistant plants. This explains why only 12.4% of the interspecific hybrids were resistant. This resistance might improve the fitness of the interspecific hybrids.

Seeds produced by the F_1 interspecific hybrids had a size close to that of the recurrent parent. The poor female fertility of the ACX hybrids may explain the low number of seeds obtained. The same results were reported from *B. napus–Diplotaxis erucoides* hybrids crossed to rapeseed (Delourme et al. 1989). Of the two types of interspecific hybrids, seed production was higher when *B. napus* was the recurrent parent. This agrees with previous observations where the number of seeds set was greater when the higher chromosome number species was used as the recurrent parent in backcrosses (Bing et al. 1991). Characterization of the seeds produced is in progress.

In comparison to the number of F_1 interspecific hybrids produced by Kerlan et al. (1992) using in-vitro methods, less ACAd and more ACRr hybrids were obtained in our experiments. For interspecific crosses between *B. napus* and *B. adpressa*, hand pollination at the early bud stage, followed by embryo rescue, allowed more hybrids to be obtained, giving a better yield than spontaneous pollination on opened flowers under natural conditions. It is likely that the limiting factor was early embryo abortion rather than the pollination itself. This was not the case for the crosses between *B. napus* and *R. raphanistrum*. By using in-vitro methods, only a few wild parents were employed, and all the variability present in the population could not be exploited. Furthermore, the total number of pollinated flowers was much higher and pollination occurred at different stages after anthesis under the natural conditions. Although the in-vitro techniques have created new opportunities for interspecific hybridization in *Brassiceae*, spontaneous pollination using a male-sterile parent has the advantage of requiring no elaborate facilities to produce seeds.

This work completes the studies initiated by Kerlan et al. (1992) on the safety assessment of gene dispersal from transgenic rapeseed to weeds. The F_1 interspecific hybrids produced were vigorous and well adapted to natural conditions, but some difficulties arose for the BC_1 seed production, particularly with the diploid species as the recurrent parent. It seems that it is difficult to return to the diploid level, which is in agreement with the results of Bing et al. (1991). Even if that difficulty could be overcome, gene introgression will depend on chromosome rearrangements in the 2x genome.

Male-sterile lines will be extensively used for rapeseed F_1 hybrid variety production. We have demonstrated that interspecific crosses can occur using male-sterile rapeseed. However, we may expect that the pollen competition due to the co-cultivation of a male-fertile rapeseed variety will result in rare pollinations

involving wild species, except where the female parent flowers earlier than the male parent.

The introgression of agronomic characters, such as blackleg resistance, would be of interest to breeders. This is linked to the ability of the weed genome to recombine with the *B. napus* genome. Some chromosome rearrangements could be expected between Rr and AC, but would be more difficult with the Ad genome. However, it would be of interest to study the possible genetic factors which may control homoeologous chromosome pairing in the *B. adpressa* population.

This work will be continued, always under natural conditions, in order to determine whether it is possible to obtain recombinant genomes, characterized by marker genes, with a diploid structure, on one hand, and with the amphidiploid *B. napus* structure, on the other hand.

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