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Assessment of interspecific hybridization between transgenic oilseed rape and wild radish under normal agronomic conditions

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Abstract In order to assess the hybridization rate between oilseed rape and wild radish under normal agronomic conditions, three 1-ha field experiments were performed. In each case, wild radish plants were transplanted at different densities in the middle, the border, or the margin of the herbicide-tolerant oilseed rape field. Among the 189084 seedlings obtained from seeds harvested on wild radish plants, only one herbicide-tolerant interspecific hybrid (RrRrAC, 2n = 37) was characterized from seeds harvested on an isolated plant growing in the margin of the field. Thus, for the wild radish total harvest, with a 95% confidence limit, the frequency of interspecific hybrids was assessed to range from 10^{-7} to 3.10^{-5}. Interspecific hybrids were detected in all cases among the smallest seeds with a diameter less than 1.6 mm harvested on oilseed rape, but the highest frequency was obtained from oilseed rape close to wild radish plants growing as clusters in the border or the margin of the field. Most hybrids had the expected triploid genomic structure (ACRr, 2n = 28) except for four amphidiploids (AACCRrRr, 2n = 56) and one hybrid from a wild radish unreduced gamete (ACRrRr, 2n = 37). Among the 73847 seedlings observed on the oilseed rape total harvest, the frequency of interspecific hybrids was assessed to range from $2.10^{-5}$to 5.10^{-4}, with a 95% confidence limit. The results are discussed with regard to the type of oilseed rape variety used and the characteristics of the interspecific hybrids.

Key words Transgenic oilseed rape · Wild radish · Hybridization rate · Agronomic conditions

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Introduction

Dispersal of transgenes in the environment through interspecific hybridization between transgenic crops and their wild relatives is a major concern. So, in a first step, several research programs have been developed to assess the possibility of gene flow between species. In a second step, the frequency of gene flow has to be investigated because the risk is dependant upon the probability of this event.

This question is particularly relevant for oilseed rape (*Brassica napus* L., AACC, 2n = 38) as it is a partially allogamous species with a numerous wild relatives present in the cultivated areas. Initial studies have been developed from herbicide-tolerant varieties as this trait is easy to analyse on large populations. Co-cultivation of oilseed rape with a weed frequent in northern European countries, field mustard (*Brassica rapa* syn *Brassica campestris*), which is one of the two progenitors of oilseed rape, revealed a high frequency of interspecific hybridization (Jorgensen and Andersen 1994; Bing et al. 1996). With weeds less related to oilseed rape, wild mustard (*Sinapsis arvensis* L., SarSar, 2n = 18), hoary mustard (*Hischfeldia incana* (L.) Lagrèze Fossat, AdAd, 2n = 14) and wild radish (*Raphanus raphanistrum* L., RrRr, 2n = 18), hybridization rates were assessed under optimal conditions using as female either male-sterile oilseed rape or isolated weedy plants with a high oilseed rape pollen pressure. Because of the frequency of interspecific hybridization between oilseed rape and wild radish (Eber et al. 1994; Baranger et al. 1995; Darmency et al. 1998) and the possibility of gene exchanges through recombination (Kerlan et al. 1993), this plant model has been chosen for further studies. From F_1 oilseed rape-wild radish interspecific hybrids, several generations were analysed under optimal field conditions (Chèvre et al. 1997, 1998). However, an assessment of hybridization rates between the two species under normal agronomic conditions was needed. The present paper reports the results obtained from seeds harvested on wild radish and on oilseed rape growing in normal field conditions.

Materials and methods

Plant materials

The oilseed rape (*B. napus* L.) variety used, named 'Synergy Basta', is a winter-type varietal association containing 80% of a male sterile (Ogu-INRA cytoplasmic male sterility) F_1 hybrid with two copies at the hemizygous stage of the transgene and 20% of a pure line homozygous for two copies of the transgene as pollinator. This pure line was provided by AGREVO. The transgene is the *pat* gene which confers tolerance to a broad-range herbicide, glufosinate-ammonium (commercial names, Basta or Liberty).

The wild radish (*R. raphanistrum* L.) populations were locally collected at Rennes and Dijon (France)

Field design

The experiments were carried out at Rennes (West of France, Brittany) in 1996 and at Dijon (East of France, Burgundy) in 1996 and 1997 with the agreement of the French Committee for Molecular Engineering (CGB).

One hectare of 'Synergy Basta' was sown in autumn. A Basta treatment was applied at the 3–4 leaf stage.

Wild radish plants were grown in the greenhouse in Jiffy pots and then transplanted in the fall in the oilseed rape field after herbicide treatment at different densities:

(1) within the field (W): either as isolated plants i.e. one plant/20 m^2 (IW) on 1000 m^2 with three replicates or in groups of two plants/m^2 (GW) on 50 m^2 with three replicates,
(2) in the border (B) of the field : either as isolated plants i.e. one plant each per 10 m (IB) or in 40-plant groups (GB) on two 10-m lines at Rennes, and 20 plants on two 5-m lines at Dijon with three replicates,
(3) in the margin of the field (M), 3 m away from the border of the field: either as isolated plants i.e. one plant each per 10 m (IM) or in 40-plant groups (GM) on two 10-m lines at Rennes, and 20 plants on two 5-m lines at Dijon with three replicates.

Observations and harvesting data

The beginning of the flowering period of each of the wild radish plants, as well as the beginning, the middle and the end of the oilseed rape flowering period, were observed.

Wild radish plants were harvested plant by plant and the number of pod portions per plant was assessed. We checked that each portion contained one seed.

Oilseed rape seeds were harvested in the trial performed in Rennes by a combine in the replicates of the field corresponding to the different locations of the wild radish plants i.e. 3 × 1000 m^2 (IW), 3 × 50 m^2 (GW), 3 × 30 m^2 (GB and GM). Seeds were separated by sieving in three grading groups : > 1.6 mm, 1 ≤ ∅ < 1.6 mm and < 1 mm. The percentages of seed weight in each category was assessed from 1.5 kg of the harvest in each replicate and the weight of 1000 seeds was observed for each grading group.

Analysis of the harvested seeds

In greenhouse, de-hulled wild radish seeds were obtained after breaking pods and were sown either in vermiculite (Dijon) or in Petri dishes and transplanted in compost (Rennes). All the seeds harvested in Dijon were sown in the greenhouse. For the Rennes trial, only the seeds harvested on IW plants that produced less than 100 seeds were analysed under greenhouse conditions, as well as samples of the smallest oilseed rape seeds (diameter < 1 mm) harvested close to wild radish plants in the IW, GW, GB and GM situations.

Under field conditions, the number of seeds sown was assessed by the seed weight.

For the wild radish trial, when a plant produced less than 900 seeds, they were hand sown. The others were machine sown with a maximum of 100 seeds per m^2. The total area of the trial was 1.3 ha. The number of emerged seedlings was assessed by counting all the plants in the hand-sown plots.

For the oilseed rape trial, all the seeds were machine sown, those with a diameter less than 1 mm at a density of 14 kg/ha (30 g/22 m^2) and those with a diameter between 1 and 1.6 mm at the density of 7 kg/ha (15 gr/22 m^2). The total surface of the oilseed rape field was 1.5 ha. The number of emerged plants was assessed either on the whole plot when there were few plants or on 2 linear m (corresponding to 1 m^2) in the other plots.

Hybrid characterization

Basta treatment

In the greenhouse, seedlings obtained from seeds harvested on wild radish or oilseed rape and cuttings of the putative hybrids were treated at the 3–4 leaf stage by spraying a 1% Basta (200 g/l of glufosinate) solution and the number of resistant plants was observed 2-weeks later.

Under field conditions, five Basta treatments were applied on wild radish plants at 4 l/ha to take into account the successive seedling emergence periods and the number of resistant plants was assessed.

Morphological characters

As most of the seedlings obtained from seeds harvested on oilseed rape were Basta tolerant, the criteria used under field conditions for hybrid identification was the duration of the flowering period as interspecific hybrids flower longer than oilseed rape because of their poor female fertility.

Cytogenetic studies

From previous studies (Eber et al. 1997), a linear regression was established between the chromosome number observed from root tips and flow cytometry data. So cytometric analyses were performed from leaves of all the putative hybrids to assess their chromosome number as described by Eber et al. (1997). 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.

PCR analysis

DNA was extracted from young leaves of hybrids identified from seeds harvested on oilseed rape according to the method of Doyle and Doyle (1990). The primers specific for the Ogu-INRA cytoplasm and the conditions of PCR amplification were as described by Tinchant et al. (1997).

Statistic analysis

The expected maximum frequency of hybrids in the samples is calculated assuming a binomial distribution with a probability of $P = 0.05$.

Results

Observations of the flowering period and harvest

Wild radish grew well in Rennes, but they were rather small at Dijon because of the calcareous soil and a cold

Fig. 1 Cumulative frequency of wild radish plant flowering in Rennes growing within the field (*IW*, *GW*) and in the border or the margin of the field (*IB*, *GB*, *IM*, *GM*); (→) start of the oilseed rape flowering period *S*, 50% of oilseed rape plants flowered *F*

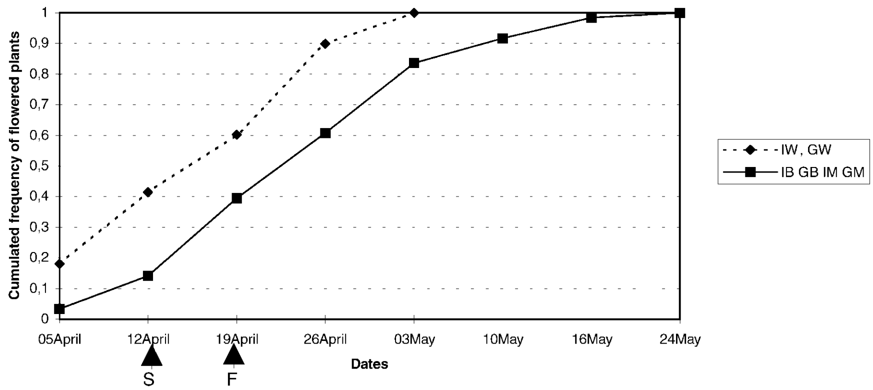

Table 1 Characterization of the seeds harvested on oilseed rape: percentages of the weight in each grading group and weight of 1000 seeds according to the location of the harvest, in the middle of the field close to isolated (IW) or grouped (GW) wild radish plants, in the border (GB) or the margin (GM) of the field close to groups of wild radish plants

Origin	Ø ≥ 1.6 mm		1 ≤ Ø < 1.6 mm		Ø < 1mm	
	Weight %	Weight per 1000 seeds in g	Weight %	Weight per 1000 seeds in g	Weight %	Weight per 1000 seeds in g
IW	87.69	3.86	5.15	1.90	7.16	0.10
GW	92.24	4.48	3.81	2.46	3.95	0.09
GB	92.57	4.14	3.16	1.65	4.26	0.14
GM	92.71	3.72	2.47	1.45	4.82	0.11

winter. The wild radish and oilseed rape started to flower at the beginning and middle of April, respectively (Fig. 1). Wild radish plants growing at the border or the margin of the field had a larger vegetative development than those in the middle of the field and, so, flowered later (Fig. 1). The end of the oilseed rape flowering period was observed at the beginning of June. A good overlap between the flowering periods was observed in all the situations (Fig. 1).

At both locations, wild radish plants growing within the field were the smallest. Consequently, the mean number of seeds per plant was lower from plants harvested in the middle of the field than from those harvested in the border or in the margin of the field: in Rennes, on average, 340 and 5400 seeds/plant, respectively, and in Dijon 3–310 seeds/plant, respectively. The number of seeds per plant ranged from 0 to 2640 and from 1 to 26500, in the middle and in the border or the margin of the field, respectively.

Seeds were harvested on oilseed rape in the Rennes trial. The largest seeds, with an average weight for 1000 seeds of 4.0 g, represented the majority of the harvest, 87.7–92.7% (Table 1). From previous data, interspecific hybrids were supposed to be present among the smallest seeds. They represented 2.5–7.2% of the total harvest (Table 1). Their average weight for 1000 seeds was assessed as 1.9 g, and 0.1 g for the seeds with a diameter between 1.6 mm and 1 mm, and with a diameter less than 1 mm, respectively (Table 1).

Characterisation of wild radish seeds

From the 63484 de-hulled seeds harvested on 468 plants, the germination rate observed in greenhouse ranged from 50 to 86%. Among the 43666 herbicide-treated seedlings, none of them were herbicide resistant i.e. no interspecific hybrid was observed.

Under field conditions, the percentage of germination assessed in the hand-sown plots was 16.4. Due to the successive seedling emergence, the first Basta treatment was late so several plants were too old and remained partially green. These 346 plants were checked by flow cytometry. Only one was detected to be a hybrid (Table 2). It was harvested on an isolated wild radish plant growing in the margin of the field which produced 327 seedlings per 1995 seeds harvested. With a 95% confidence limit, the frequency of hybrids among the seedlings observed from seeds harvested on wild radish plants growing in an isolated situation in the margin of the field was assessed to range from 4.10^{-4} to 9.10^{-2} (Table 2). In the other situations, no interspecific hybrids were observed and the assessed frequencies are reported in Table 2. From the total of 189084 seedlings obtained from 951622 seeds, the expected frequency of hybrids, from the total harvest, whatever the location of the wild radish plants, was assessed to range from 10^{-7} to 3.10^{-5}.

The interspecific hybrid had a normal vegetative development with a morphology intermediate between the two parental species. Its chromosome number, 37 (RrRrAC), indicated that it was produced from an unreduced gamete of wild radish and a reduced gamete

Table 2 Number of hybrids produced by wild radish harvested in three experimental trials (1 ha each) where a number of wild radish plants were planted as isolated (I) or in groups (G), within (W), on the border (B) or on the margin (M) of a Basta-resistant oilseed rape field. Hybrids were identified as being resistant to the herbicide. The 95% confidence limits (95% CL) of the percentage of hybrid are given according to the number of seedlings sprayed with herbicide in the greenhouse (Dijon and Rennes experiments) or in the field (Rennes experiment). (* = estimated number)

Location	Condition	No. of plants harvested	No. of seeds sown	No. of seedlings	No. of hybrids	95% CL of the % of hybrids
Rennes	IW	113	43403	7921	0	0–0.05
Dijon 96		105	150	75	0	0–4.8
Dijon 97		70	38	30	0	0–10.9
Rennes	GW	301	100800	16500*	0	0–0.02
Dijon 96		30	188	161	0	0–2.2
Dijon 97		30	338	218	0	0–1.4
Rennes	IB	13	39170	6400*	0	0–0.06
Dijon 96		54	3321	1796	0	0–0.2
Dijon 97		15	129	128	0	0–4.0
Rennes	GB	108	310082	50800*	0	0–0.007
Dijon 96		30	960	793	0	0–0.05
Dijon 97		30	932	718	0	0–0.6
Rennes	IM	16	38759	6300*	1	0.0004–0.09
Dijon 96		59	53877	37400*	0	0–0.01
Dijon 97		9	1746	1244	0	0–0.3
Rennes	GM	118	357729	58600*	0	0–0.006

Table 3 Number of hybrids harvested on oilseed rape plants close to wild radish growing either in the middle of the field as isolated plants (IW) or in groups (GW), or in the border (GB) or the margin (GM) as clusters (* = estimated values)

Condition	Seed diameter	No. of seeds sown*	No. of seedlings*	No. of hybrids	95% CL of the % of hybrids
IW	Ø < 1 mm	4333400	2653	2	0.009–0.27
GW	Ø < 1 mm	9000100	4221	1	0.0006–0.13
GB	1 ≤ Ø < 1.6 mm	274600	32912	4	0.003–0.031
	Ø < 1 mm	4296400	3715	7	0.076–0.39
GM	1 ≤ Ø < 1.6 mm	279600	26532	4	0.004–0.039
	Ø < 1 mm	7854900	3814	5	0.039–0.31

of oilseed rape. Its average meiotic behavior was 3.58 univalents + 15.42 bivalents + 0.42 trivalents + 0.33 quadrivalents, established from 12 pollen mother cells. Its male fertility was assessed to be 6.5%.

Characterisation of oilseed rape seeds

As it was established from our previous data that interspecific hybrids are present among the smallest seeds (Eber et al. 1994), only seeds with a diameter less than 1.6 mm were examined.

Samples of the 1006 smallest seeds (∅ <1 mm) were treated in the greenhouse. The percentage of germination ranged from 31 to 67%. Among the 605 seedlings obtained, nine interspecific hybrids were observed by a flow cytometry only among seeds harvested on oilseed rape close to wild radish plants growing in the border (GB) or the margin of the field (GM).

The smallest seeds (∅ <1 mm) harvested on oilseed rape growing close to IW, GW, GB and GM radish plants were analysed under field conditions. According to the data obtained in greenhouse i.e. observation of interspecific hybrids harvested in GB and GM situations, oilseed rape seeds with a diameter between 1 and 1.6 mm harvested in these two locations were also sown. These two situations corresponded to the highest wild radish pollen concentration on oilseed rape mother plants. The percentage of germination was low for the smallest seeds

Table 4 Characterization of the interspecific hybrids harvested on oilseed rape close to wild radish plants growing either in the middle of the field as isolated plants (IW), in groups (GW) or in the border (GB), and in the margin (GM) of the field as clusters. – missing data; R: Basta resistant, S: Basta susceptible

Origins	No. of plants	2n	Basta	Cytoplasm
IW	1	28	–	–
	1	28	R	Ogu-INRA
GW	1	37	R	Ogu-INRA
GB	1	28	–	–
	6	28	R	Ogu-INRA
	3	28	S	Ogu-INRA
	1	56	S	**Oilseed rape**
GM	2	28	R	Ogu-INRA
	4	28	S	Ogu-INRA
	2	56	R	Ogu-INRA
	1	56	S	Ogu-INRA

(∅ <1 mm), ranging from 0.04 to 0.08 whereas the larger seeds (1.6 mm ≤ ∅ < 1 mm) had a better ability to germinate i.e. 9–12%. At the maturity of oilseed rape, 1240 plants still in flower were harvested and analysed by flow cytometry. Among them, 14 interspecific hybrids were detected. Interspecific hybrids were found in all the locations but a higher frequency was observed from seeds harvested on oilseed rape plants close to wild radish growing as clusters in the border or the margin of the field (Table 3). Among the 73847 plants obtained from around 26 million of the smallest oilseed rape seeds har-

Table 5 Meiotic behaviour and male fertility of interspecific hybrids harvested on oilseed rape mother plants (* ranges are indicated in parenthesis)

Cytoplasm	No. of plants	2n	Cells	Meiotic behaviour				% of male fertility
				Univ.	Biv.	Triv.	Quadriv.	
Ogu-INRA	3	28	54	12.19 (8–16)*	7.80 (6–10)		0.05 (0–1)	0
Ogu-INRA	1	**56**	16	6.38 (4–10)	24.44 (23–26)	0.25 (0–1)		0
Oilseed rape	1	**56**	30	7.30 (4–13)	23.37 (20–26)	0.57 (0–1)	0.07 (0–1)	85

vested in the different locations around the wild radish plants, the expected frequency of hybrid plants ranged from 2.10^{-5} to 5.10^{-4}, with a 95% confidence limit. However these smallest seeds represented only 7.89% of the total harvest.

The characteristics of the 23 interspecific hybrids obtained are reported in Table 4. Eighteen of them had the expected genomic structure i.e. 2n = 28 (ACRr). However, one hybrid obtained from an unreduced gamete of wild radish (2n = 37, ACRrRr) and four amphidiploid plants (2n = 56, AACCRrRr) were also produced. They had a morphology similar to that of oilseed rape except for the 2n = 37 hybrid which was intermediate between the two parental species. Cuttings were performed from interspecific hybrids, when possible, for cytoplasm and Basta resistance analyses. Among the 21 plants further characterized, only one Basta resistant plant had an oilseed rape cytoplasm and thus was produced on a fully fertile oilseed rape mother plant (Table 4). Among the 20 hybrids with an Ogu-INRA cytoplasm, 60% were Basta resistant (Table 4); this segregation was not significantly different from the one expected from an hemizygous oilseed rape mother plant with two copies on the transgene. The meiotic behavior of triploid and amphidiploid hybrids was established (Table 5). The average of bivalents in ACRr (2n = 28) hybrids shows that chromosome pairing can occur between the different genomes. Mainly bivalents were formed in amphidiploid plants but the high frequency of univalents and of multivalents indicated chromosomal instability. Whatever the genomic structure, interspecific hybrids with an Ogu-INRA cytoplasm were sterile whereas the amphidiploid with an oilseed rape cytoplasm was highly male fertile (Table 5).

Discussion

Under our field conditions, as frequently observed in oilseed rape production areas, wild radish plants flowered at the same period as oilseed rape. We also observed that interspecific hybrids can be produced on both species.

The competition with oilseed rape was lower for wild radish plants growing in the border or the margin of the field. Consequently, these latter had a larger vegetative development, flowered later and produced more seeds than the plants growing within the field in spite of the large variability observed among the population analyzed. High variation exists also for the percentages of germination due to the conditions in the greenhouse or in the field, and also for dormancy. This character is highly variable among weed populations and it has been shown that interspecific hybrids may have an intermediate behavior between the parents (Chadoeuf et al. 1998). As observations were performed without treatments to break dormancy in the greenhouse, and at one date after several herbicide treatments under field conditions, the rate of hybridization was calculated from emerged seedlings. As seed dormancy depends on the mother tissues, it could represent an underestimate of the actual rate because some hybrids could remain dormant. Only one hybrid was observed from the 3-ha experiments. It belongs to seeds harvested on an isolated plant in the margin of the field, which was less than 3×10^{-5} of the wild radish seeds produced in the field. This result is in agreement with the hypothesis that this situation corresponds to the highest probability to obtain interspecific hybrids on wild radish, as already described by Darmency et al. (1998). In fact the self-incompatibility described in this species (Sampson 1967) preventing selfing and the high pressure of oilseed rape pollen on isolated plants are the most favorable situations for interspecific hybridization. In addition, plants of wild radish growing in the border and the margin of the field produced more flowers, so that this situation also corresponded to the higher probability of interspecific hybridization. This situation is not very different from what can actually occur in farmer's fields since our 3-ha experiments were designed to have different patches of plant density and the overall plant number was less than 600. Indeed, weed populations are not spread evenly, but rather display spatial heterogeneity (Cousens and Mortimer 1995), so that some plants can occur completely isolated amongst oilseed rape. The mean density of wild radish in France ranges from 2.3 to 7.7 plant/m^2 in herbicide-free control fields, according to the region involved (Barralis 1977). An efficient herbicide reduces this amount by 95–99%, which results in the same number of plants remaining in the field as in our experiment.

We analyzed only the smallest seeds harvested on oilseed rape in agreement with the results of several authors (Eber et al. 1994; Baranger et al. 1995) who indicated that interspecific hybrids are present only among seeds with a diameter less than 1.6 mm. The low fertility of F_1 interspecific hybrids (Eber et al. 1994; Baranger et al. 1995; Chèvre et al. 1997; Darmency et al. 1998) has, as a consequence, a longer period of flowering than fully fertile plants. This criterion allowed us to identify several interspecific hybrids. A total of 23 hybrids were detected from the 1-ha experiment. The highest frequency of interspecific hybrids was observed in situations in which the wild radish pollen concentration was the highest, as expected. Seeds harvested on oilseed rape were not tested at Dijon because the wild radish pollen pressure appeared to be too low. However, no significant difference was observed between seeds harvested on oilseed rape plants close to wild radish populations growing in the border of the field or in the margin (Table 3). This result indicated that the pollen pressure from the crop was not sufficient to prevent interspecific hybridization. It was confirmed by harvesting seeds on male-sterile oilseed rape plants growing in the margin of the field, i.e. both 5 m away from the field and 5 m away from any wild radish plants; 0.9–5.8 interspecific hybrids per oilseed rape plant were observed (data not shown), indicating that pollen flow from oilseed rape was a limiting factor and resulted in large number of hybrids produced by isolated plants. Interspecific hybrids were observed in all the situations analysed. It is likely that the presence of more than 80% of male-sterile oilseed rape plants in the variety can explain this result, as confirmed by the observation that 95% of the interspecific hybrids had the same cytoplasm as the male-sterile oilseed rape i.e. the Ogu-INRA cytoplasm. Because these male-sterile plants were hemizygous for two copies of the transgene, 38% of the interspecific hybrids were Basta susceptible, which somewhat limited the transgene spread.

Most of the interspecific hybrids had the expected triploid genomic structure, ACRr (2n = 28). Their meiotic behavior was similar to the one already described by Eber et al. (1994) with a high frequency of pairing, indicating that chromosome exchanges may occur between the different genomes. However, amphidiploid plants with both genomes at the diploid stage can also be produced as shown by Baranger et al. (1995). The male fertility is clearly under control of the cytoplasm as only the amphidiploid with an oilseed rape cytoplasm was fully fertile. In spite of the high frequency of univalents and multivalents, these hybrids had a good female fertility producing a large number of seeds, especially the one on oilseed rape cytoplasm (data not shown). This latter plant was observed under greenhouse conditions and it is likely that similar hybrids were not detected under field conditions because they were as fertile as oilseed rape. This observation suggests a possible under-estimation of interspecific hybridization from oilseed rape seeds. The possible occurrence of new fertile weeds from such hybrids and their fitness have to be studied. The hybrids with 2n = 28 (ACRr) or 2n = 56 (AACCRrRr) had a morphology close to that of oilseed rape whereas the two 2n = 37 (ACRrRr) hybrids were intermediate between the two species whatever the cytoplasm, wild radish or Ogu-INRA. Their genomic structure is explained by the production of wild radish unreduced gametes, already described among Crucifer species by Heyn (1977). In spite of the presence of the wild radish genome at the diploid stage, their male and female (data not shown) fertility was low.

The interspecific hybridization rate was higher from seeds harvested on oilseed rape than from the ones collected on wild radish plants. This result is not in agreement with that obtained after reciprocal hand crosses which indicated no difference (Kerlan et al. 1992). The structure of the oilseed rape variety used, containing 80% of male-sterile plants, could explain this data; the pollen pressure from oilseed rape was 5-times lower than in a fully fertile field and the 80% of male-sterile plants caught the first pollen available. Hence, the interspecific hybridization rate was most likely under-estimated on wild radish and over-estimated on oilseed rape. Further field experiments from a fully fertile oilseed rape variety, herbicide-tolerant in a cultivated area spontaneously contaminated by wild radish, will be performed, the impact of current agricultural practices on the occurrence of interspecific hybrids having already been assessed in a multi-year and multi-crop monitoring study (Champolivier et al. 1999).

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