

Opportunities for gene transfer from transgenic oilseed rape (*Brassica napus*) to related species

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Before novel transgenic plant genotypes are grown outside containment facilities and evaluated under field conditions, it is necessary to complete a risk assessment to consider the possible consequences of that release. An important aspect of risk assessment is to consider the likelihood and consequences of the transgene being transferred by cross-pollination to related species, including other crops, weeds and ruderal populations. The purpose of this report is to review the literature to assess the ease with which *Brassica napus* can hybridize with related species. The evidence for hybridization is considered at three levels: a) by open pollination, b) by hand pollination and c) by the use of *in vitro* ovule and embryo rescue techniques; and also examines the fertility and vigour of the F₁, F₂ and backcross generations. Four species are reported to hybridize with *B. napus* by open pollination: *B. rapa* and *B. juncea* using fully fertile parents; and *B. adpressa* and *R. raphanistrum* using a male-sterile *B. napus* parent. Seventeen species are reported to form hybrids (including the four species above) with *B. napus* when pollination is carried out manually. At least 12 of these species were unable to form F₂ progeny, and eight were unable to produce progeny when the F₁ was backcrossed to one of the parental species. Many factors will influence the success of hybridization under field conditions, including: distance between the parents, synchrony of flowering, method of pollen spread, specific parental genotypes used, direction of the cross and the environmental conditions. Even where there is a possibility of hybridization between *B. napus* and a related species growing in the vicinity of a release, poor vigour and high sterility in the hybrids will generally mean that hybrids and their progeny will not survive in either an agricultural or natural habitat.

Keywords: *Brassica napus*; oilseed rape; transgenic plants; interspecific hybridization; gene transfer; risk assessment

Introduction

With the opportunities now provided by recombinant DNA techniques and plant transformation, the gene-pool for many plant species has been extended outside the boundaries defined by sexual incompatibility. It is likely that a wide variety of transgenic plants will be developed within the next two decades, and that transgenic oilseed rape (*Brassica napus* L.) will be at the forefront of this development. The most promising transgenic plant genotypes will be evaluated outside a containment greenhouse under field conditions following regulatory procedures and legislation developed or adapted for the purpose (USDA, 1991; EEC Directive 90/220, 1990). To date, there have been more than 400 small-scale field experiments with transgenic plants, and over 80 of these have been with oilseed rape (Chasseray and Duesing, 1992; Dale *et al.*, 1993).

There is wide acceptance of the need for regulations governing the release of transgenic plants, principally because it is now possible to introduce into crop plants, genes (from unrelated plants, microbes and animals) that were previously inaccessible through conventional plant breeding (OECD, 1986; National Research Council USA, 1989; Royal Commission, 1989). As a result, the range of genotypes and phenotypes that can now be produced may potentially fall outside the experience of traditional genetics and plant breeding. It is also likely that genes will be inserted for the production of novel kinds of products for industrial or pharmaceutical use (Dale *et al.*, 1993).

Before transgenic plants can be grown under field conditions, it is necessary to carry out a risk assessment to determine the consequences of releasing a particular plant species containing specific transgenes (Health and Safety Commission, 1992; OECD, 1990; USDA, 1991). An important part of that risk assessment is to estimate the likelihood of transgenes being transferred by cross-pollination to sexually compatible species, including other crops,

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weeds and ruderal plants. If there is the possibility for hybrids to form, it is necessary to assess the consequences of transfer of the transgenes, and whether they could have any adverse human or environmental impact (Dale, 1992, 1994). For example, what would be the consequences of a herbicide resistance gene being transferred to a weed species, or could insect resistance confer a selective advantage to ruderal plant species?

Successful hybrid formation depends not only on the sexual compatibility of the crop plant and recipient species, but the two species must also flower at the same time, share the same insect pollinator (if insect-pollinated) and be at a sufficiently close distance for the transfer of viable pollen (Scheffler *et al.*, 1993). If the recipient species is sexually compatible with oilseed rape, the consequences of transfer of the transgenes will depend on the sexual fertility of the hybrid progeny, their vigour and sexual fertility in subsequent generations, or their ability to propagate vegetatively (Singh *et al.*, 1990).

Sexual compatibility in the present context depends on the degree of relatedness between oilseed rape and its relatives. Many studies of *Brassica napus* have involved the use of interspecific hybrids in experiments to define the taxonomic relationships within the Brassicaceae family (Karpechenko, 1922; Pearson, 1928; Sinskaia, 1928; Morinaga, 1929a, b) and to study chromosome pairing and behaviour (Catchside, 1934; U, 1935; Haga, 1938; Sikka, 1940; Mizushima, 1950a; Harberd, 1972, 1976). Interspecific hybridizations have also been attempted to evaluate the potential for gene transfer (Heyn, 1977; Ringdahl *et al.*, 1987), and several genes have been successfully transferred into *B. napus* from other species (Lammerink, 1970; Johnston, 1974; Chiang *et al.*, 1979; Roy, 1984). Some recent studies have been aimed at evaluating the potential for transfer of transgenes between oilseed rape and specific recipient species (Bing *et al.*, 1991; Chevre *et al.*, 1992; Kerlan, 1992a, b; Lefol, 1993; Eber *et al.*, 1994).

Several earlier reviews have described cytological and taxonomic relationships within the Brassicaceae, and include data on sexual compatibility among some members of the family (Manton, 1932; Yarnell, 1956; Heyn, 1977; Harberd, 1976; Harberd and McArthur, 1980; Prakash and Hinata, 1980). Since these reports, many more studies have extended this information, and some very recent experiments have included transgenic plants with marker genes that are useful for monitoring transgene transfer.

The objective in this paper is to present a review of the current state of knowledge on the sexual compatibility between oilseed rape and its related species, to evaluate the likelihood of the transfer of transgenes to related species, and to present the information in a form useful to researchers and regulators interested in, or required to assess, the potential for gene transfer from transgenic *Brassica napus* by hybridization.

Results and discussion

Methods of ovule culture and embryo rescue, developed over several decades, have extended the range of species that can be sexually hybridized with *Brassica napus*. Many of the new hybrid combinations made in this way would not occur naturally because of barriers within the plant that prevent normal embryo or endosperm development. While these techniques provide an important method for transferring genes that would not otherwise be accessible, the ease with which hybrids are formed when *in vitro* methods are used is not indicative of the probability of similar hybrids occurring by cross-pollination under natural outdoor conditions (Table 1). The hybrids formed can, however, provide valuable information on the chromosome pairing behaviour between weakly homologous genomes, and facilitate the evaluation of hybrids between *B. napus* and related species to assess their potential to survive and persist in nature.

The most relevant data for assessing the likelihood of gene transfer is that from reports of naturally occurring hybrids found in areas where the two parental species are found growing in the same area. These reports are rare and often anecdotal. Stace (1991) cites a naturally occurring hybrid between *Brassica napus* and *Brassica rapa* which has been named *B. x harmsiana*. The hybrid had a chromosome number of $2n = 29$, and was sterile. Stace also reported that the hybrid occurred "sporadically in crops of *B. napus* when exposed to pollination by *B. rapa*".

There are several reports (Table 2) of hybrids resulting from open pollination between *B. napus* and a related species grown in field trials specially designed to obtain hybrids (Kajanus, 1917; Mackay, 1973) or to measure the potential for hybrid formation (U and Nagamatsu, 1933; Heyn, 1977; Bing *et al.*, 1991; Lefol, 1993). The majority of the literature describes attempts at interspecific and intergeneric hybridization by manual cross-pollination. The results may give a biased estimate of the ease or success of each hybridization, because successful hybridizations are likely to be reported more often than unsuccessful attempts. With this proviso, the data provide valuable information on the potential for gene transfer from *B. napus* to its related species.

In all the reports reviewed, the number of hybrid seeds per pollination was never as high for interspecific as for intraspecific hybridization, but as would be expected, the closer the relationship between the parental genotypes, the greater the probability of successful hybridization. The degree of relatedness between species is conventionally based on a taxonomic classification developed using cross-compatibility, morphology and degree of chromosome pairing. Early studies by Schulz (1919) were continued by many other researchers, and much of this work has been reviewed by others including Hedge (1976) and Prakash

and Hinata (1980). U (1935) assigned genome designations to each of the *Brassica* species he studied, and he devised a triangle to describe the relationships between them. The relationships he proposed were further studied and amended by other researchers including Olsson (1949) and Mizushima (1980). Harberd (1972, 1976) grouped the species into cytodesmes which he defined as all species that share a common chromosome complement. If two species had the same chromosome number and could form a fertile vigorous hybrid, they were classed as being derived from the same cytodesme. There is good agreement between this earlier work, and later studies using chemical analysis of seed proteins (Vaughan, 1977), differences in protein composition (Gatenby and Cocking, 1978), isozyme studies (Takahata and Hinata, 1986) and RFLP (restriction fragment length polymorphism) analyses of chloroplast or genomic DNA of numerous members of the Brassicaceae family (Erickson *et al.*, 1983; Song *et al.*, 1988; Warwick and Black, 1991; Song and Osborn, 1992).

Hybrids produced by manual cross-pollination are possible between *Brassica* species with at least one genome in common. As shown in Table 2, *B. napus* (genome AACC) hybrids are most often successful in crosses with its progenitor species, *B. rapa* (Genome AA; also known as *B. campestris*). Although hybridization is more successful when *B. rapa* is the pollen parent, hybrids have been produced using *B. rapa* as either the female or male parent. Many of the seeds produced were non-viable when *B. rapa* was the female parent, and in all progeny from crosses in this direction there was reduced pollen fertility (0% to >50%). Not all seeds produced were hybrid, so the hybrid status needed to be confirmed. Second generation progeny (F₂) and backcross (BC) progeny can be produced by manual pollination. Using *B. napus* as the female parent, a small amount of seed has reportedly been produced in field experiments by open pollination (U and Nagamatsu, 1933; Heyn, 1977; Bing *et al.*, 1991). Hybrids from *B. napus* × *B. rapa* crosses have been used in breeding programmes (Grabiec, 1971; McNaughton and Ross, 1978) particularly in Japan (Shiga, 1970; Namai *et al.*, 1980).

Crosses between *B. napus* and its other progenitor species, *B. oleracea*, are more difficult to obtain, and have been more successful when *B. oleracea* is the male parent. Only Becker (1950) and Röbbelen (1966) have reported obtaining viable hybrid plants when diploid *B. oleracea* was used as the female parent (Table 2). Röbbelen observed reduced pollen fertility in the hybrid progeny, and although it was possible to produce F₂ progeny, the F₂ seeds were often shrivelled and germinated poorly. To our knowledge there are no reports of hybrids being produced by open pollination.

Brassica juncea (AABB) and *B. carinata* (BBCC) are both tetraploid species that share one genome with *B.*

napus. Hybrids between *B. napus* and *B. juncea* have been reported by at least ten researchers, and there is one report of a small number of hybrid seeds being produced under field conditions by open pollination in mixed stands of *B. napus* and *B. juncea* (Bing *et al.*, 1991). Hybrids using *B. carinata* have also been produced by manual pollination, and were more successful when *B. napus* was the female parent. F₂ or BC progeny could be produced in most cases; however, male fertility was always low and often no or only few seeds were produced.

Hybrids using *B. nigra* (BB) as the male parent have also been reported, and Bing *et al.* (1991) describes obtaining one F₁ plant when *B. nigra* was the female parent. The hybrid plants were characterized by low fertility, and limited success in producing F₂ or BC progeny. Open pollination under field conditions was unsuccessful (Bing *et al.*, 1991). Although *B. napus* and *B. nigra* have no genomes in common, bivalent pairing of chromosomes has been observed, and it has been suggested that the B and C genomes are partially homologous (Mizushima, 1980).

Heyn (1977) reported viable F₁ hybrids in crosses using *B. fruticulosa* or *B. tournefortii* as the male parent. The F₁ plants formed only vestigial anthers without pollen and did not set any seeds after pollination by either parental species.

Hybrids between *B. napus* and *B. adpressa* have previously been unsuccessful; however, Chevre *et al.* (1992) and Lefol (1993) reported obtaining hybrids by planting male-sterile *B. napus* and fully fertile *B. adpressa* in adjacent rows in a field trial and allowing open pollination. A small number of hybrid seeds were produced, and the F₁ plants from these exhibited varying degrees of infertility, but some seeds were produced when the hybrids were backcrossed to *B. adpressa* (Eber *et al.*, 1994).

Within the same subtribe as *Brassica* are several other genera including *Diplotaxis*, *Erucastrum*, *Eruca*, *Raphanus* and *Sinapis*. Hybridization with a range of *Diplotaxis* species has been attempted, but only *D. erucoides*, *D. muralis* and *D. tenuifolia* have produced viable F₁ progeny (Table 2 and 3). Ringdahl *et al.* (1987) described the F₁ plants as male-sterile, but were able to produce a few seeds by backcrossing the plants to *B. napus*. Heyn (1977) reported obtaining one F₁ plant in a cross using *D. tenuifolia* as the male pollen parent. Heyn (1977) also reported producing 11 F₁ hybrids using *Eruca sativa* as the male parent. The fertility of the hybrids produced by Heyn was not reported.

It has been proposed that the AA genome (*B. rapa*) and the RR genome of *Raphanus spp.* originated from a common ancestor (Harberd, 1976; Mizushima, 1980; Song *et al.*, 1988; Warwick *et al.*, 1992). Nevertheless, manual hybridization is usually unsuccessful. The hybrid status of the progeny has been confirmed only in crosses

Table 1. Attempted hybridizations, using ovule or embryo culture, between *Brassica napus* and other members of the tribe Brassiceae

Scientific name	Normal chromosome number & genome designation	Parent ^a	Number of embryos or ovules cultured	F ₁ hybrid plants produced	F ₂ or BC progeny produced ^b	Reference ^c
<i>Brassica adpressa</i> Boiss. (<i>Hirschfeldia incana</i>) (L.) Lagrèze-Fossat	2n = 2x = 14 B _{ad} B _{ad}	M	262 ovaries	8	?/0 ^d	Kerlan, 1991, 1992a
		F	1117 ovaries	15	?/0 ^d	Kerlan, 1991, 1992a
		M	157 ovaries	6	?/?	Lefol, 1993
<i>Brassica carinata</i> Braun	2n = 4x = 34 BBCC	F	22 ovaries	0	0/0	Lefol, 1993
		M	50 embryos	3	yes/?	Rousselle, 1983
		F	17 embryos	2	yes/?	Rousselle, 1983
<i>Brassica gravinae</i>	2n = 4x = 40 GGGG	M	7033 ovules	2038	yes/yes	Sacristan, 1986
		F	33 ovaries	1	0/yes	Nanda Kumar, 1989
		F	20 ovaries	0	0/0	Nanda Kumar, 1989
<i>Brassica juncea</i> (L.) Czern.	2n = 4x = 36 AABB	M	15	8	yes/?	Rousselle, 1983
		F	64	18	yes/?	Rousselle, 1983
		M	194	1-4/ovary	yes/?	Bajaj, 1986
		F	142	1-4/ovary	yes/?	Bajaj, 1986
		M	360 ovules	144	yes/?	Bajaj, 1986 ^e
		F	564 ovules	244	yes/?	Bajaj, 1986
		M	152	90	yes/?	Bajaj, 1986
		F	126	92	yes/?	Bajaj, 1986
		M	7033 ovules	2038	yes/yes	Sacristan, 1986
		F	?	yes	yes/yes	Prakash, 1988
<i>Brassica nigra</i> (L.) Koch	2n = 2x = 16 BB	M	1	1	yes/?	Rousselle, 1983
		F	14	3	yes/?	Rousselle, 1983
		M	56 ovules	4	?/?	Diederichsen, 1988
		F	14 ovules	0	0/0	Diederichsen, 1988
		M	?	1	0/yes	Jahier, 1989
		M	325	11	?/0 ^d	Kerlan, 1992a
		F	916	0	0/0	Kerlan, 1992a
<i>Brassica oleracea</i>	2n = 2x = 18 CC	F	11	0	0/0	Takeshita, 1980
		M	?	0-2.64 plts/poll.	?/?	Ayotte, 1987
		M	332	79	few/yes	Quazi, 1988
		F	89	21	few/yes	Quazi, 1988
		M	433	41	?/0 ^d	Kerlan, 1992a
		F	1030	4	?/0 ^d	Kerlan, 1992a
		M	111	20	yes/?	Rousselle, 1983
<i>Brassica rapa</i> L.	2n = 2x = 20 AA					

Scientific name	Normal chromosome number & genome designation	Parent ^a	Number of embryos or ovules cultured	F ₁ hybrid plants produced	F ₂ or BC progeny produced ^b	Reference ^c
(<i>Brassica campestris</i>)		F	58	14	yes/?	Rousselle, 1983
<i>Diplotaxis eruroides</i> (L.) DC	2n = 2x = 14	F	432 ovaries	66	?/yes	Delourme, 1989
<i>Diplotaxis siifolia</i> G. Kunze	2n = 2x = 20	M, F	134	yes	2/?	Batra, 1990
<i>Enarthrocarpus lyratus</i> (Forsk.) DC.	2n = 2x = 20	F	?	yes	yes/yes	Gundimeda, 1992
<i>Erucastrum gallicum</i>	2n = 2x = 30	M	?	0	0/0	Batra, 1989
<i>Raphanus raphanistrum</i> L.		F	11	3	?/?	Batra, 1989
	2n = 2x = 18	M	368	3	?/0 ^d	Kerlan, 1992a
		F	583	9	?/0 ^d	Kerlan, 1992a
		M	308	1	?/?	Lefol, 1993
			ovaries			
		F	165	1	?/?	Lefol, 1993
			ovaries			
<i>Raphanus sativus</i>	2n = 2x = 18	M	61 ovules	1	?/?	Takeshita, 1980
		F	4 ovules	0	0/0	Takeshita, 1980
		F	?	yes	?/?	Paulmann, 1988
		M	84 ovaries	24	?/?	Luo, 1989
<i>Sinapis alba</i>	2n = 2x = 24	M, F	268	yes	?/0	Ripley, 1990
		M	?	0	0/0	Mathias, 1991
		F	56 ovules	2	?/?	Mathias, 1991
		M	586	11	?/yes	Chevre, 1991
		F	412	9	?/yes	Chevre, 1991
		M	890	0	0/0	Lelivelt, 1993
		F	412	6	?/yes	Lelivelt, 1993
<i>Sinapis arvensis</i> L. (<i>Brassica kaber</i>)	2n = 2x = 18	M	50 ovules	6	yes/yes	Inomata, 1988
		M	?	0	0/0	Mathias, 1991
		F	19 ovules	1	?/?	Mathias, 1991
		M	808	18	?/0 ^d	Kerlan, 1992a
		F	732	0	0/0	Kerlan, 1992a
		M	1126	25	?/?	Lefol, 1993
			ovaries			
		F	852	1	?/?	Lefol, 1993
			ovaries			
<i>Sinapis pubescens</i> L.	2n = 2x = 18	M	103	5	0/0	Inomata, 1991
			ovaries			
		F	57 ovaries	0	0/0	Inomata, 1991

The normal number of chromosomes and the genome designation (if known) is given.

^aThe species listed in the table were used as the male (M) or female (F) parent in crosses with *B. napus*.

^bNumbers of progeny produced in the F₂/ or from a backcross (BC) to one of the parents (in most cases *B. napus*). The ? indicates that progeny were not reported; yes indicates that progeny were produced, but no number was given.

^cEach reference is identified by the first author and year of publication.

^dBackcross progeny were produced using ovule culture.

^eThe paper by Bajaj *et al.*, 1986 contained results from two experiments that could not be combined.

Table 2. Attempted hybridizations between *Brassica napus* and other members of the tribe Brassiceae

Scientific name	Normal chromosome number & genome designation	Parent	Number of flowers pollinated	F ₁ hybrid plants produced	F ₂ or BC progeny produced	Reference	
<i>Brassica adpressa</i> Boiss. (<i>Hirschfeldia incana</i>) (L.) Lagrèze-Fossat	2n = 2x = 14 B _{ad} B _{ad}	M	118	0	0/0	Kerlan, 1992a	
		F	149	0	0/0	Kerlan, 1992a	
<i>Brassica carinata</i> Braun	2n = 4x = 34 BBCC	M	open poll. ^{a,b}	yes	?/yes	Lefol, 1991, 1993; Chevre, 1992	
		M	55	1	yes/?	U, 1935	
		F	35	3 seeds	0 plants	0/0	U, 1935
		M	26	yes ^c	??	??	Heyn, 1977
		M	~ 512	4% seed set	yes/yes	yes/yes	Roy, 1980
		F	~ 83	1% seed set	??/0	??/0	Roy, 1980
		M	128	13	yes/yes	yes/yes	Aslamyousuf, 1982
		F	119	0	0/0	0/0	Aslamyousuf, 1982
		M, F	?	yes	yes/yes	yes/yes	Fernandez-Escobar, 1988
		M, F	?	yes	yes/yes	yes/yes	Fernandez-Serrano, 1991
<i>Brassica fruticulosa</i>	2n = 2x = 16 FF	M	246	2	0/0	Heyn, 1977	
<i>Brassica gravinae</i>	2n = 4x = 40 GGGG	M	109	0	0/0	Nanda Kumar, 1989	
		F	61	0	0/0	Nanda Kumar, 1989	
<i>Brassica juncea</i> (L.) Czern.	2n = 4x = 36 AABB	M	47	0.3–3 seed /pod	??/?	Pearson, 1928	
		F	45	7–18 seed /pod	??/?	Pearson, 1928	
		F	?	yes	yes/yes	yes/yes	Sinskaia, 1928
		M	?	7	??/?	??/?	Morinaga, 1929a, 1929c
		M, F	?	yes	yes/?	yes/?	Morinaga, 1934
		M	?	yes	few/?	few/?	Sasaoka, 1930
		F	?	yes	few/?	few/?	Sasaoka, 1930
		M, F	?	yes	??/?	??/?	Olsson, 1949
		M	99	343 ^c	??/?	??/?	Heyn, 1977
		M	26	11	??/?	??/?	Heyn, 1977
		F	?	?	yes/yes	yes/yes	Roy, 1978
		F	~ 132	yes	yes/yes	yes/yes	Roy, 1980
		M	~ 900	yes	yes/yes	yes/yes	Roy, 1980
		M	422	23/100 poll.	??/yes	??/yes	Yamagishi, 1982
F	105	~ 22 seeds	??/yes	??/yes	Mathias, 1985		
F	?	yes	??/yes	??/yes	Prakash, 1988		
F	1021	4103	yes/?	yes/?	Bing, 1991		
F	6208	204	??/?	??/?	Bing, 1991		
M	469 plants ^a	469 plants ^a	5	??/?	Bing, 1991		

Scientific name	Normal chromosome number & genome designation	Parent	Number of flowers pollinated	F ₁ hybrid plants produced	F ₂ or BC progeny produced	Reference
		M, F	?	yes	yes/yes	Fernandez-Serrano, 1991
<i>Brassica nigra</i> (L.) Koch	2n = 2x = 16 BB	M	?	0	0/0	Pearson, 1928
		M, F	?	0	0/0	Sinskaia, 1928
		M	18	7	0/?	Heyn, 1977
		M	?	0	0/0	Diederichsen, 1988
		F	?	0	0/0	Diederichsen, 1988
		M	742	7	few/few	Bing, 1991
		M	710	0	??	Bing, 1991
			plants ^a	confirmed		
		F	973	1	0/1	Bing, 1991
		F	1188	0	0/0	Bing, 1991
			plants ^a			
		M	320	0	0/0	Kerlan, 1992a, 1992b
		F	531	0	0/0	Kerlan, 1992a, 1992b
<i>Brassica oleracea</i> L.	2n = 2x = 18 CC	M	?	0	0/0	Sutton, 1908
		F	?	0	0/0	Sutton, 1908
		M	?	0	0/0	Pearson, 1928
		F	?	0	0/0	Pearson, 1928
(<i>Brassica alboglabra</i> , Bayley)		M, F	?	0	0/0	Sinskaia, 1928
		M	908	3	yes/?	U, 1935
		F	81	2 seed 0 plants	0/0	U, 1935
		M	5873	3209 seed 184 plants	?/13	Roemer, 1935
		F	?	0	0/0	Roemer, 1935
		M	134	0	0/0	Calder, 1937
		F	306	0	0/0	Calder, 1937
		M	1965	670 seed ^e	??	Becker, 1950
		F	1470	13 seed ^e	??	Becker, 1950
		M	8705	32 plants	?/0	Hoffmann, 1958
		M	92	31	??	Röbbelen, 1966
		F	139	21	??	Röbbelen, 1966
		M	> 100	1	?/yes	Honma, 1976
		M	6142	3	0/yes	Chiang, 1977
		F	106	0	0/0	Chiang, 1977
		M	~ 1,000	0	0/0	McNaughton, 1978
		F	11	0	0/0	Takeshita, 1980
		M	542	0.9/100 poll.	?/yes	Yamagishi, 1982
		M	279	0	0/0	Kerlan, 1991, 1992a, 1992b
		F	364	0	0/0	Kerlan, 1991, 1992a, 1992b
<i>Brassica rapa</i> L.	2n = 2x = 20 AA	M	?	yes	many/0	Sutton, 1908
(<i>Brassica campestris</i>)		F	?	yes	few/0	Sutton, 1908
		M	38	437	many ^f /?	Kajanus, 1917
		F	19	13	few ^f /?	Kajanus, 1917

<i>Scientific name</i>	<i>Normal chromosome number & genome designation</i>	<i>Parent</i>	<i>Number of flowers pollinated</i>	<i>F₁ hybrid plants produced</i>	<i>F₂ or BC progeny produced</i>	<i>Reference</i>
		M	?	yes	many/?	Nelson, 1927
		F	?	yes	few/?	Nelson, 1927
		M	135	7-11 seed	??	Pearson, 1928
		F	41	0-6 seed	??	Pearson, 1928
(<i>Brassica chinensis</i> L.)		M, F	?	1.7/ silique	yes/yes	Sinskaia, 1928
(<i>Brassica pekinensis</i>)		M, F	?	6.6/ silique	yes/yes	Sinskaia, 1928
		M	?	yes	yes/yes	Morinaga, 1929b
		F	?	yes	yes/yes	Morinaga, 1929b
		M	6349 plants ^a	4%	??	U, 1933
		F	3520 plants ^a	0	0/0	U, 1933
		M	140	2255 seed 75% hybrid	yes/?	U, 1935
		F	119	1569 seed 21% hybrid	yes/?	U, 1935
		M	715	1505 seed 24 plants	?/yes	Roemer, 1935
		M	100	228 seed ^c	??	Calder, 1937
		M	251	1830 seed ^c	??	Calder, 1937
		F	251	1452 seed ^c	??	Calder, 1937
		F	? ^a	25 plants	??	Calder, 1937
		M	753	2409 seed ^c	??	Becker, 1950
		F	482	433 seed ^c	??	Becker, 1950
		M	?	yes	??	Mizushima, 1950a
		M	1225	4294 seed ^c	??	Olsson, 1949
		F	116	55 seed ^c	??	Olsson, 1949
(<i>B. pekinensis</i>)		M	17484	709	yes/yes	Hoffmann, 1958
(<i>B. pekinensis</i>)		M	116	709 seed 689 hybrids	yes/?	Jahr, 1962
		F	189	389 seed 105 hybrids	yes/?	Jahr, 1962
		M	121	317	??	Röbbelen, 1966
		F	343	0	0/0	Röbbelen, 1966
		F	5 plants	?	yes/0	Lammerink, 1970
		M	?	yes	yes/yes	Nwankiti, 1971
		M	9 plants ^g	38.9 seed /plant	yes/?	Mackay, 1973
		F	9 plants ^g	5.2 seed /plant	yes/?	Mackay, 1973
		M	?	yes	?/yes	Johnston, 1974
		M	420	3761 ^c	??	Heyn, 1977
		M	8	39	0/?	Heyn, 1977
		M	?	yes	yes/yes	Namai, 1977
		M, F	?	yes	yes/yes	McNaughton, 1978
		M	15 plants	yes	?/yes	Beversdorf, 1980
		F	15 plants	yes	?/yes	Beversdorf, 1980
		?	?	yes	?/yes	Gowers, 1982
		M	358	252/100 poll.	?/yes	Yamagishi, 1982
		M, F	?	yes	yes/yes	Mero, 1984

Scientific name	Normal chromosome number & genome designation	Parent	Number of flowers pollinated	F ₁ hybrid plants produced	F ₂ or BC progeny produced	Reference
		F	133	1242	??	Bing, 1991
		F	2129	21	??	Bing, 1991
			plants ^a			
		M	130	1938	??	Bing, 1991
		M	790	21	??	Bing, 1991
			plants ^a			
<i>Brassica tournefortii</i>	2n = 2x = 20	M	80	55	0/0	Heyn, 1977
	TT					
<i>Diplotaxis assurgens</i> (Del.) Gren	2n = 2x = 18	F	566	0	0/0	Ringdahl, 1987
<i>Diplotaxis crassifolia</i> (Forsk.) Boiss	2n = 2x = 26	F	790	0	0/0	Ringdahl, 1987
<i>Diplotaxis cretacea</i> Kotov	2n = 2x = 22	F	265	0	0/0	Ringdahl, 1987
<i>Diplotaxis eruroides</i> (L.) DC	2n = 2x = 14	F	561	3	0/yes	Ringdahl, 1987
	D _c D _e					
<i>Diplotaxis harra</i> (Forsk.) Boiss	2n = 2x = 13	F	94	0	0/0	Ringdahl, 1987
<i>Diplotaxis ibicensis</i> (F.) Quer	2n = 2x = 16	F	99	0	0/0	Ringdahl, 1987
<i>Diplotaxis muralis</i>	2n = 4x = 42	F	285	31	0/yes	Ringdahl, 1987
<i>Diplotaxis pitardiana</i> Maire	2n = 2x = 22	F	821	? ^h	0/0	Ringdahl, 1987
<i>Diplotaxis sietiana</i> Maire	2n = 2x = 16	F	267	0	0/0	Ringdahl, 1987
<i>Diplotaxis siifolia</i> G. Kunze	2n = 2x = 20	M	74	0 ⁱ	0/0	Batra, 1990
		F	78	0	0/0	Batra, 1990
		F	841	0	0/0	Ringdahl, 1987
<i>Diplotaxis tenuifolia</i> (L.) DC	2n = 2x = 22	M	7	1	? ^d /?	Heyn, 1977
	D ₁ D ₁	F	654	0 ^j	0/0	Ringdahl, 1987
<i>Diplotaxis tenuisiliqua</i> Del.	2n = 2x = 18	F	715	0 ^j	0/0	Ringdahl, 1987
<i>Diplotaxis viminea</i>	2n = 2x = 20	F	415	0	0/0	Ringdahl, 1987
<i>Diplotaxis virgata</i>	2n = 2x = 18	F	683	0	0/0	Ringdahl, 1987
<i>Eruca sativa</i>	2n = 2x = 22					
	EE	M	88	11	? ^d /?	Heyn, 1977
<i>Erucastrum gallicum</i>	2n = 2x = 30	F	138	0	0/0	Batra, 1989
		M	?	0 ⁱ	0/0	Batra, 1989
<i>Raphanus raphanistrum</i> L.	2n = 2x = 18	M	148	0	0/0	Kerlan, 1991, 1992a, 1992b
		F	132	0	0/0	Kerlan, 1991, 1992a, 1992b
		M	open poll. ^{a,b}	yes	?/yes	Chevre, 1992
						Lefol, 1993
<i>Raphanus rugosum</i>	2n = 2x = 16	M	14	10	? ^d /?	Heyn, 1977
<i>Raphanus sativus</i>	2n = 2x = 18	M	69	0	0/0	Becker, 1950
	RR					
		F	40	0	0/0	Becker, 1950
		?	?	yes	0/0	McNaughton, 1978
		M	30	0	0/0	Takeshita, 1980
		F	12	0	0/0	Takeshita, 1980
		M	5752	10	0/?	Luo, 1989
<i>Sinapis alba</i> L.	2n = 2x = 24	M	?	0	0/0	Nelson, 1927

Scientific name	Normal chromosome number & genome designation	Parent	Number of flowers pollinated	F ₁ hybrid plants produced	F ₂ or BC progeny produced	Reference
<i>(Brassica hirta)</i>		F	?	0	0/0	Nelson, 1927
		M	?	0	0/0	Pearson, 1928
		F	?	0	0/0	Pearson, 1928
		M, F	?	0	0/0	Olsson, 1949
		M	128	0	0/0	Becker, 1950
		M	148	2	? ^d /?	Heyn, 1977
		M	~ 7000	0 ¹	0/0	Lelivelt, 1993
<i>Sinapis arvensis</i> L.	2n = 2x = 18 S _{ar} S _{ar}	F	?	0 ¹	0/0	Lelivelt, 1993
		M	?	0	0/0	Pearson, 1928
<i>(Brassica kaber)</i>		M, F	?	0	0/0	Sinskaia, 1928
		M, F	?	0	0/0	Olsson, 1949
		M	?	1	0/?	Mizushima, 1950a
		M	?	3 ^m	0/0	Inomata, 1988
		F	623	0	0/0	Bing, 1991
		F	7189 plants ^a	0	0/0	Bing, 1991
		M	576	0	0/0	Bing, 1991
		M	745	0	0/0	Bing, 1991
		M	82 plants ^a	0	0/0	Kerlan, 1991, 1992a, 1992b
		F	199	0	0/0	Kerlan, 1991, 1992a, 1992b

The normal number of chromosomes and the genome designation (if known) is given. Manual pollination has been used for the attempted hybridizations, unless it is stated specifically that open pollination was used. For further information on presentation see Table 1.

^aOpen-pollinated in the field.

^bMale sterile *Brassica napus* used as a female parent.

^cHybrid status determined by seed size only.

^dA few putative F₂ seed produced, but the genomic constitution and viability not reported.

^eSeed produced were not confirmed as hybrid.

^fF₂ seeds obtained by open pollination in the field.

^gPollinated by blow flies in cages.

^hTwo albino seedlings, but not confirmed as hybrids.

ⁱA few seeds produced, but all were of matromorphic origin.

^jThree weak seedlings died, but not confirmed as hybrids.

^kOne weak seedling died, but not confirmed as hybrid.

^lSeeds were produced, but none were confirmed as hybrids.

^mOne with chromosome number 2n = 28 and two with 2n = 37. A few F₂ seeds obtained by open pollination.

between *B. napus* and *R. sativus*, when *R. sativus* was used as the pollen parent (Luo *et al.*, 1989; McNaughton and Ross, 1978). The two parents belong to different taxonomic genera, but partial chromosome pairing has been observed in hybrids between the two, and also in hybrids between *B. rapa* (AA) and *R. sativus* (Harberd and McArthur, 1980; Mizushima, 1980).

Heyn (1977) reported obtaining F₁ hybrids in crosses where *R. rugosum* was the male parent. Kerlan *et al.* (1992b) attempted reciprocal manual pollinations between *R. raphanistrum* and *B. napus*, but failed to obtain any viable F₁ hybrids after making 200 manual

pollinations. Cytological examination of pollen tube growth showed that *R. raphanistrum* pollen tubes did not penetrate the *B. napus* styles. Chevre *et al.* (1992) and Lefol (1993) reported obtaining hybrid seed in a field trial similar to that described for *B. adpressa*, with adjacent rows of *R. raphanistrum* and male-sterile *B. napus*. Pollen fertility of the F₁ hybrids ranged from 0% to 30%. Backcross progeny were produced by open pollination when the F₁ was used as the female parent (Eber *et al.*, 1994).

Several members of the genus *Sinapis* were at one time classified within the genus *Brassica*, but RFLP data

indicate that the genus is more distantly related to *Brassica* than members of the genus *Raphanus* (Song *et al.*, 1990; Warwick and Black, 1991). Only Heyn (1977) reported successful hybridization with *S. alba*. There is no indication that fertile F₂ or BC progeny were produced.

Another member of the genus, *S. arvensis*, has been more intensely studied by several researchers (Table 2), but only Mizushima (1950a) and Inomata (1988) reported obtaining hybrids. In both cases, *S. arvensis* was the pollen parent. The single F₁ plant produced by Mizushima was male sterile and failed to produce progeny. Inomata reported having 3 F₁ hybrids, two of which had 37 chromosomes, indicating that two sets of chromosomes were contributed by the *S. arvensis* parent. Seeds were produced by open pollination on two of the F₁ plants. It is not known if these seeds were viable. Studies of pollen tubes show that growth usually stopped before penetrating the style (Kerlan *et al.*, 1992b). Two experiments have been reported where *B. napus* and *S. arvensis* were grown together in a field plot and allowed to open pollinate (Bing *et al.*, 1991; Lefol, 1993). Neither study produced confirmed hybrids.

Throughout the reports reviewed, several phenomena were observed repeatedly and should be considered when evaluating the success of an interspecific or intergeneric hybrid. The first is the production of matromorphic seed (U, 1935; Röbbelen, 1966; Chiang *et al.*, 1977; Heyn, 1977; Roy, 1980; Batra *et al.*, 1989) where the chromosomes of the female gamete double and a seed is produced without any chromosomes contributed by the male. Pollination of stigmas by foreign pollen has been shown to stimulate the production of matromorphic seed in the Brassiceae, so all seeds produced from interspecific hybrids should be confirmed as such. When *B. napus* is used as the female parent, there is also the possibility that some of the seeds produced will not be hybrid, but spontaneous haploids of *B. napus*. Like the progeny of interspecific hybrids, these are characterized by small seeds, small plants and low fertility (Morinaga and Fukushima, 1933; Olsson and Hagberg, 1955; Thompson, 1969; Stringam and Downey, 1973; Renard and Dosba, 1980; Eber *et al.*, 1994).

The success of an interspecific or intergeneric hybrid may be influenced by the genotypes used in the cross (Becker, 1950; Honma and Summers, 1976; Chiang *et al.*, 1977; Roy, 1980; Ayotte *et al.*, 1987). This does not necessarily imply that one genotype will be consistently successful where others have failed, but rather that one genotype may produce a few hybrids under specific conditions where others have failed completely. Becker attempted manual hybridization of field-grown *B. napus* and *B. oleracea* using ten different *B. oleracea* varieties and four different *B. napus* varieties over seven years. Generally, when *B. oleracea* was used as the female, no seeds were produced; however, in one year, two plants (of

different genotypes) produced a total of 11 seeds. He continued crossing experiments in subsequent years, but failed to obtain more seeds from either of the previous successful genotypes. However, he did obtain two more seeds from a different cross combination in the final year of the experiment.

Roy (1980) attempted to cross *B. napus* with *B. juncea* and *B. carinata* using a number of different genotypes. Some combinations of genotypes failed to produce any seeds while others were successful. The genotype used also appeared to affect the probability of success in the subsequent F₂ or backcross generations. A differential genotype response has also been noted when hybrids were produced using ovary culture/embryo rescue techniques (Ayotte *et al.*, 1987). Honma and Summers (1976) and Chiang *et al.* (1977) found that all of their successful hybrids had a *B. oleracea* parent that was itself a progeny of an intraspecific cross between two subspecies.

Hybridization is possible between *B. napus* and some members of the Brassiceae although success, even under optimum conditions, is often low. In the most successful cross combination (*B. napus* × *B. rapa*), the number of seeds per pollination (Table 2) ranged from 0 to 15 with an average of less than ten. In intraspecific crosses between *B. napus* genotypes, the number of seeds per pollination is generally 20 or greater. When the long term survival of transferred genes is taken into account, based on fertility and vigour of the F₁ hybrids, the chances of success of transferred genes becoming established in new hybrid derived populations, is even lower. Table 3 summarizes the combinations where hybrids have been reported (based on the literature reviewed in Table 2), and ranks them according to the probability that progeny (F₁, F₂ and BC) can be produced after controlled pollination. It does not indicate that these hybrids will occur under field conditions.

Persistence of hybrids through successive generations depends on their ability to survive and reproduce. In general, the fertility of F₁ hybrids was low, and male sterility was common. The fertility of hybrid plants was increased in some cases when the chromosome number was doubled (Morinaga and Fukushima, 1933; U, 1935; Türesson and Nordenskiöld, 1943; Mizushima, 1950b; Jahr, 1962; Nwankiti, 1971; Chiang *et al.*, 1977; Heyn, 1977; Chevre *et al.*, 1991). Increase in the chromosome number can occur spontaneously, either in the F₁ hybrid or in later generations, but can lead to decreased as well as increased fertility. Progeny have been generated in some cases by using the F₁ plant as the female parent, and backcrossing to one of its parental types. When one of the parents was diploid, progeny were often obtained only when *B. napus* was used as the female parent in the backcross. Because of this unilateral incompatibility, which has been reported for both the initial interspecific hybridization and subsequent generations, flow of genes is

most likely to be from related species into *B. napus*, and not the reverse (Nishiyama *et al.*, 1991).

After several generations of backcrossing under controlled conditions, plants resembling the recurrent backcross parent were obtained from an F₁ hybrid between *B. napus* and *B. rapa* (Nwankiti, 1971; Shiga, 1970). This indicates that, in theory, it might be possible to produce a hybrid plant capable of surviving (at least under agronomic cultural conditions). However, for a plant to reach this stage, the two parental species would have to flower at the same time and be close enough for pollen transfer to occur. Seeds would have to be produced that are capable of germinating and producing a plant with sufficient fertility to produce progeny. If backcrossing was required to produce the progeny, then a suitable parent would have to be available, and flowering at the same time as the F₁ plant. While the possibility can never be discounted, the evidence indicates that it would be a rare occurrence.

Conclusions

It is not possible to make simple statements to describe the precise limits of sexual barriers between *B. napus* and its related species, or of the likelihood of hybrids forming and persisting in agricultural and natural habitats. The opportunity for hybridization depends on the: physical distance between the two species, synchrony of flowering, method of pollen dissemination, specific parental genotypes used, direction of the cross, whether one parent is male-sterile, and the environmental conditions. The success of the F₁ hybrid and its progeny will be influenced by: growth vigour, fertility, ability to propagate vegetatively, ability to give viable F₂ and backcross progeny, and the ability to survive over subsequent generations. If there is the possibility of viable hybrids forming under agricultural or natural conditions, it is necessary, in a risk assessment, to consider whether the particular transgene might confer a selective advantage or be, in some way, harmful to human health or the environment (Dale, 1994).

In a review of this kind, it is necessary to rely on the available literature in determining the likelihood of initial hybridization, and the subsequent viability and fertility of the progeny. Although the experimental reports cited have been reviewed carefully, the conclusions drawn from each, inevitably, depend on the judgement of the original investigators and the rigor of their analysis. Where hybrids have been obtained from a particular species combination in several laboratories, it is reasonable to assume that the hybridization is relatively easy to perform. Where there are few reports of an unsuccessful combination, this may not accurately reflect the amount of effort devoted to achieving hybridization, because of the tendency to under-report negative results.

In using this review for specific risk assessment exer-

Table 3. Relative ranking of species by their ability to form hybrid progeny when crossed with *B. napus*^a

Species	F ₂ progeny produced	Backcross progeny produced	Rank
<i>B. rapa</i>	yes	yes	1
<i>B. juncea</i>	yes	yes	2
<i>B. oleracea</i>	yes	yes	3
<i>B. carinata</i>	yes	yes	4
<i>B. nigra</i>	? ^b	yes	5
<i>B. adpressa</i>	no	yes	6
<i>R. raphanistrum</i>	no	yes	6
<i>D. erucoides</i>	no	yes	7
<i>D. muralis</i>	no	yes	7
<i>S. alba</i>	no	no	8
<i>S. arvensis</i>	no	no	8
<i>B. fruticulosa</i>	no	no	9
<i>B. tournefortii</i>	no	no	9
<i>D. tenuifolia</i>	no	no	9
<i>E. sativa</i>	no	no	9
<i>R. rugosum</i>	no	no	9
<i>R. sativus</i>	no	no	9

Species with no reports of successful hybridization were excluded.

^aThe ranking is based on the ability of F₁ hybrids to be produced following manual pollination, and an assessment of the likelihood of the formation of F₂ and backcross progeny. Only four species are reported to have produced F₁ progeny with *B. napus* following open pollination under field conditions; *B. rapa* and *B. juncea* using fully fertile parents, and *B. adpressa* and *R. raphanistrum* using male-sterile *B. napus*.

^bBing *et al.* (1991) reported possible progeny produced.

cises it is suggested that Table 3 is consulted to determine whether there is evidence, using manual pollinations, of hybrids being formed between *B. napus* and particular species growing in the vicinity of the release site. It should be noted that there are reports of hybridization under open pollination conditions between fully fertile parents with only two species, *B. rapa* and *B. juncea*. Where the *B. napus* is male sterile, there is a greater opportunity for hybridization, but under these specific conditions, hybrids are reported for only *B. adpressa* and *R. raphanistrum*. Information on the number of independent studies there have been with particular species, details of the results obtained, and the authors of the reports, are presented in Table 2. The success of hybridization using ovule or embryo culture methods is detailed in Table 1.

While hybridization data, following the use of manual hybridization and of sophisticated *in vitro* methods to rescue hybrid embryos, give no measure of the likelihood of hybridization in nature, they do allow the risk assessors to hypothesize beyond the initial sexual barrier, and make judgements on the long-term viability and fertility of a particular hybrid. Hypothesizing in this way will become more relevant as the release of transgenic plants moves

from the small scale evaluation of novel transgenic genotypes in very specific and well-defined habitats, to the widespread international use of transgenic varieties for agricultural production in many kinds of environments.

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