REVIEW

Aspects of the biosafety of transgenic oilseed rape (*Brassica napus* L.)

PETER L. J. METZ*‡, EVERT JACOBSEN† and WILLEM J. STIEKEMA*

Department of Molecular Biology, CPRO-DLO, Wageningen, The Netherlands, †Department of Plant Breeding, Agricultural University Wageningen, Wageningen, The Netherlands

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INTRODUCTION

Genetic modification of crop plants has resulted in plants resistant against pathogens or showing improved quality. Within the coming years it is expected that more transgenic crops will be commercialized and there is little doubt that transgenic plants will significantly contribute to agriculture in the future (Dale & Irwin 1995). Calgene's Laurate oilseed rape has now full clearance from the US authorities for commercialization (APHIS/USDA 1994). However, developments in the patenting of genes, the release regulations, food labelling and consumer attitude will influence the implementation rate.

Genetically modified or transgenic plants are defined according to Stiekema & van Vloten-Doting (1991) as plants which genomes accommodate new pieces of DNA which are introduced by other procedures than sexual crossing. In spite of the fact that close similarities exist between the phenotypes of transgenic and non-transgenic crops, its application cannot simply be equalled to traditional breeding. Genetic modification makes possible the circumvention of the natural crossing-barriers between species established by evolutionary processes. Unforeseen consequences (see, for example, Maessen, 1997, this issue) may be the result and, therefore, prior to the release of these transgenic crops, their biosafety has to be assessed (Kapteijns 1993). This includes the assessment of aspects such as gene dispersal and introgression of these genes in their wild relatives via, subsequently, greenhouse experiments, small-scale field experiments followed by large-scale field trials (see, for example, Van Raamsdonk & Schouten, 1997, this issue). In this respect the Dutch government follows a 'case by case' and 'step by step' policy on the release of genetically modified transgenic plants into the environment. As starting point the 'yes, provided that . . .' principle is handled, which means

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‡Correspondence author.

that it is allowed to produce and grow genetically modified plants, provided no additional ecological and toxicological negative side effects occur. The OECD 'familiarity principle' (OECD 1993a)—biotechnology is acceptable if no additional negative aspects are involved compared to conventional methods—and the criterion of 'substantial equivalence' (OECD 1993b)—transgenic food is acceptable as long as it meets already accepted threshold values for toxic components—express the same policy in an international context.

In this review aspects concerning the biosafety of transgenic oilseed rape (*Brassica napus* L. ssp. *oleifera* (Metzg.) Sinsk.) will be discussed. From a biosafety point of view oilseed rape is interesting as it is a partially allogamous crop with an average outcrossing rate between 15% and 45% (Rakow & Woods 1987; Becker *et al.* 1992). Furthermore, transgenic oilseed rape will be at the forefront in the field release of transgenic crops and their commercialization (Dale 1993; Dale *et al.* 1993; OECD 1993; APHIS/USDA 1994; Ward 1994). In a report published by the OECD (1993c) it is stated that by far the biggest part of the field trials involving transgenic crops is done with oilseed rape. Thus, oilseed rape is suitable as model plant for biosafety studies.

BIOSAFETY OF TRANSGENIC OILSEED RAPE

In this paper a short historical perspective is given to illustrate the general appearance of oilseed rape. Furthermore, the reproduction system and taxonomy and cytogenetics of oilseed rape will be discussed. These determine pollen spread and hybridization potential, respectively, which are important factors concerning biosafety of transgenic oilseed rape. Finally, the effect of the transgene involved which is an important factor in biosafety studies of transgenic crops in general is briefly mentioned.

History

Ancient Sanskrit writings from 2000–1500 BC are considered to be the earliest references to oilseed rape in India (Singh 1958; cited in Appleqvist 1972). The Mediterranean area is suggested to be the centre of origin of this species which has been cultivated for thousands of years in Asia and the Indian subcontinent (Renard *et al.* 1993). It is assumed that both oilseed rape and turnip (*B. campestris*, syn *B. rapa*) have been cultivated as oil crops in those European countries where olive trees and poppy were unknown (Schiemann 1932). In one of his reports Linnaeus (1745; cited in Appleqvist 1972) remarks the overgrow of barley and rye by rape, reducing the grain yields. He wrote 'No herb can be more easily planted than this one, which hardly can be eradicated from the fields, and thus none could be planted to greater advantage for oil production.'

The abundant growth in grain fields may have led to domestication of rape. In the 17th and 18th centuries, methods to suppress the weed flora were not or not often applied. Eventually, rape got the upper hand, outcompeted the major (grain) crops and having favourable properties for humans was harvested. In addition to this, crop plants were adapted to growing conditions of man-made habitats (De Wet & Harlan 1975). This way, new culture forms such as oilseed rape and turnip originated. These crops are called secondary crops (Zeven 1975, 1977), in contrast to primary crops such as rye and barley.

Oilseed rape is domesticated fairly late. A reason for this may be the presence of a thioglycoside which hampered the use of the seeds for human consumption, because it
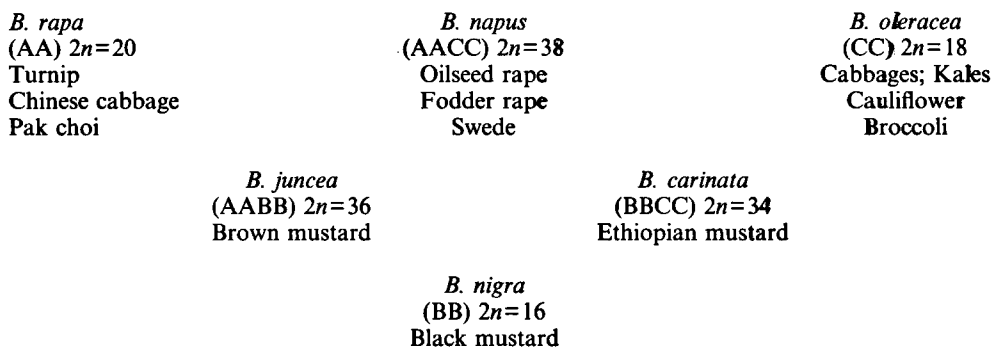
B. rapa
(AA) 2n=20
Turnip
Chinese cabbage
Pak choi

B. napus
(AACC) 2n=38
Oilseed rape
Fodder rape
Swede

B. oleracea
(CC) 2n=18
Cabbages; Kales
Cauliflower
Broccoli

B. juncea
(AABB) 2n=36
Brown mustard

B. carinata
(BBCC) 2n=34
Ethiopian mustard

B. nigra
(BB) 2n=16
Black mustard

Fig. 1. 'U-triangle' representing the genomic relations among *Brassica* species (redrawn from U 1935).

causes goitre (Johnston & Jones 1966). It is noteworthy that in Canada, presently one of the world's largest oilseed rape producing countries, commercial growing of rape seed only started in 1942 (Ohlson 1972).

The history of oilseed rape shows that it is a generally occurring species which, providing the climatic circumstances are suitable, can spread easily and can even become a threat to other crops as noted by Linnaeus. These are relevant characteristics in relation to biosafety.

Taxonomy and cytogenetics

The genus *Brassica* belongs to the family of *Cruciferae*. U (1935) designed a so-called triangle, clearly describing the genomic relationship of some *Brassiceae* (Fig. 1). More recently, Random Amplified Polymorphic DNA (RAPD) analysis confirmed the classical 'U triangle' relationship between diploid and allotetraploid *Brassica* species. The corners of this triangle are the three diploid species: *B. rapa* or turnip (AA, $n=10$), *B. nigra* or black mustard (BB, $n=8$) and *B. oleracea* or cabbage (CC, $n=9$). *B. napus* is an allotetraploid species derived from the hybridization of *B. rapa* and *B. oleracea* with the genome constitution AACC ($2n=38$). On the two other sides of the triangle *B. juncea* or Indian or brown mustard (AABB, $2n=36$) and *B. carinata* or Abyssinian mustard (BBCC, $2n=34$) are denoted. These allopolyploid species are, in contrast to the self-incompatible diploid species, self-fertile and show preferential chromosome pairing. They are probably arisen from natural hybridization in which $2n$ gametes were involved. The cytogenetic relations between the diploid species were confirmed by nuclear DNA content (Verma & Rees 1974), DNA analysis (Erickson *et al.* 1983) and by genome specific chromosome markers (Hosaka *et al.* 1990). It is noteworthy that the A-genome of *B. rapa* is common to the three commercial oilseed species; *B. juncea, B. napus* and *B. rapa*.

Based on the results of studies of the chromosome pairing in the pachytene of the meiosis of amphihaploid F_1s, the basic haploid chromosome number of the diploid *Brassica*s is hypothesized to originate from that of an ancestor with $n=6$ (Röbbelen 1960). Based on nuclear Restriction Fragment Length Polymorphisms (RFLPs), Song *et al.* (1990) proposed a new hypothesis, according to which the most ancient group contains species with $n=7$. Duplication and triplication of certain chromosomes might have led to the current basic numbers. Mutual crossability of the three diploid species is still allowed in very low percentages (U 1935; Olsson 1960; Röbbelen 1966). Due to

the independent evolution of these species their chromosome structure has altered so much that they are no longer homologous, but have become homoeologous, resulting in limited and hampered chromosome pairing.

Based on the two hypotheses about the origin of the three diploid species and the allotetraploids and on a study with genome-specific DNA markers (Hosaka *et al.* 1990), to a certain extent homoeologous chromosome pairing due to homologous chromosome parts might be expected. However, comparing established cultivars and resynthesized oilseed rape Lydiate *et al.* (1993) showed that only in the latter *B. rapa* chromosomes relatively frequently paired with *B. oleracea* chromosomes. Analysis with RFLP markers revealed 15% homoeologous recombination. This demonstrates the presence of controlled chromosome pairing in established *B. napus*. However, translocations which probably result of homoeologous recombination in the allotetraploid genome of oilseed rape suggest that domesticated *B. napus* is unable to control chromosome pairing completely (Sharpe *et al.* 1995). Due to genetic linkage maps based on RFLP markers which have been generated for *B. oleracea* (Slocum *et al.* 1990), *B. napus* (Landry *et al.* 1991; Lydiate *et al.* 1993; Parkin *et al.* 1995) and *B. rapa* (Song *et al.* 1991; Chyi *et al.* 1992) synteny studies are now in progress (Lydiate 1996).

Research comparing chloroplast-DNA of several *Brassica* species, for instance *B. napus*, *B. rapa* and *B. oleracea*, indicates that probably a third unknown *Brassica* species, through introgression, is involved in the origin of oilseed rape (Palmer *et al.* 1983). The chloroplast-DNA from two of the three accessions of *B. napus* studied strongly differed from that of both parents, while the chloroplast-DNA of the third accession corresponded with that of *B. rapa*. These results suggest that *B. napus* has been arisen more than once in different ecological areas.

Hybridization of *B. napus* with species in other genera is also reported (McNaughton & Ross 1978; Kerlan *et al.* 1992; Lelivelt 1993; Scheffler & Dale 1994). However, using RAPD markers it was shown that *Raphanus sativus* and *Sinapis alba* were distinct from the *Brassica* taxa (Demeke *et al.* 1992). RAPD markers are similar to RFLP markers for estimating intraspecific genetic relationships, while estimating inter-specific genetic relationships RAPD markers may be less reliable than RFLP markers (Thormann *et al.* 1994). In a later section, the ability of *B. napus* to cross with different *Cruciferae* species and the possible ecological impact involved, are discussed in more detail.

Reproductive system

Flower biology. The rapeseed flower consists of four half-spreading sepals, four diagonally standing petals almost twice as long as the sepals, six stamens, of which two shorter outer standing, and a superior ovary with two parietal placentas (Heywood *et al.* 1993). This flower structure is typical for *Cruciferae*. At the basis of the two shorter stamens oilseed rape flowers contain two functional nectaries, while at the basis of both pairs of long stamens two non-functional nectaries are located (Downey *et al.* 1980). The oilseed rape flowers are brightly yellow coloured and the presence of the nectaries make them very attractive to bees. Studies with petal-less *B. rapa* mutants showed that, in this case, pollination was not reduced (Brunel *et al.* 1994). The floral arrangement of the *Brassiceae* is a corymbiform raceme. The relative position of the buds to the open flowers on a raceme makes it possible to distinguish between flowering plants of *B. napus* and *B. rapa* (Clapham *et al.* 1958; Downey *et al.* 1980). In *B. napus* still-closed buds

overtop the opened flowers, while in *B. rapa* the opened flowers overtop the, compared to *B. napus* more compact, bud cluster.

Flowering begins at the lowest part of the raceme and from there upward. Both the onset of flowering and the duration of the flowering period vary and depend on weather conditions, particularly temperature. Flowers open very early in the morning and opening is completed at about 9 a.m. From 3 days before to 3 days after opening of the flowers the stigma is receptive (Mohammad 1935). Pollen from most of the oilseed rape cultivars can be stored for up to a year at low temperature ($-20°C$) and desiccation over silica gel without any adverse effects on seed yield and embryo development (Brown & Dyer 1991). Dry pollen of oilseed rape measures about $20 \times 40\,\mu m$ (Wodehouse 1935).

Pollination and fertilization. In contrast to both its parental species, in which a sporophytic incompatibility mechanism in the stigma prevents self-fertilization, oilseed rape is a predominantly self-pollinated crop with an average outcrossing rate between 15% and 45% (Rakow & Woods 1987; Becker *et al.* 1992). Environmental factors can greatly influence these outcrossing rates. Furthermore, among flowers at different positions on the plant the outcrossing rate varies from 11% at the top to 39% at the bottom of the plant (Becker *et al.* 1992).

Neither insect visits nor wind are a prerequisite for successful self-pollination of oilseed rape, although wind does stimulate this process. However, in greenhouse experiments, plants that were standing in an air flow gave more seed set than plants that were not (Williams 1978; Williams *et al.* 1986) and under large-scale commercial production conditions under which insect pollination is of secondary importance wind is the main pollinating agent (Downey *et al.* 1980; Timmons *et al.* 1995). For self-incompatible *B. rapa* both insect and wind pollination are important.

Because pollen is the main vector through which transgenes escape (Ellstrand & Marshall 1985; Den Nijs 1989) in the framework of an EU-funded biosafety project field experiments were performed to study pollen dispersal of transgenic oilseed rape (De Greef 1990). It was found that the frequencies of transgene dispersal rapidly decrease with the distance to the source of the transgenes. At four meters the outcrossing frequency was already diminished to less than 1 in a 1000 (De Greef 1990), while Paul *et al.* (1995) observed less than 0·001% outcrossing at 1 m from the pollen source. Paul *et al.* (1995) also detected limited gene dispersal (0·012%) which frequency was strongly influenced by the immediately adjacent plants. The very limited transfer was found to be characteristic for pollen transfer by bees, but as oilseed rape is also wind-pollinated the strong influence of immediately surrounding plants was not expected. Scheffler *et al.* (1993), who extensively studied pollen dispersal from transgenic oilseed rape, also found a sharp decline in outcrossing frequency of 0·02% at 12 m. In addition, they did not find a directional effect due to wind or insect activity. However, evaluating the effectiveness of 200- and 400 m isolation distances for small-scale trials of transgenic oilseed rape, the frequency of hybrids detected at 400 m was 10 times greater than estimated in the earlier study (Scheffler *et al.* 1993) for plants 47 m from the pollen donor sources (Scheffler *et al.* 1995). A major difference in the two studies was the area of non-transgenic plants. In the second study donor and target plots were smaller and separated by greater distances. Therefore, bees may have been forced to forage in more than one plot regardless of the greater distance. If they could collect a full load of pollen and nectar in a small area, in and around the donor plot, they would not forage in more distant

areas (Scheffler et al. 1995). Surrounding the transgenic plot with a trap or buffer crop of the same species that can clean emigrating pollinators of transgenic pollen and provide a sufficient source of nectar and pollen so pollinators are not inclined to forage more distant sites was an effective strategy for reducing the escape of transgenic pollen (Morris et al. 1994; Scheffler et al. 1995). However, neither barren zones nor trap crops would guarantee total isolation.

Besides that of Scheffler et al. (1995), there are also other reports of long-distance pollen flow from B. napus at distances of 360 to 2000 m (Downey et al. 1980; Downey & Bing 1990; Timmons et al. 1995, 1996). Exposing emasculated and subsequently self-pollinated plants to airborne pollen from an isolated field of another oilseed rape cultivar yielded 3·7% (5/135) inter-cultivar hybrids at 360 m (Timmons et al. 1996). Downey & Bing (1990) found 2·1, 1·1 and 0·6% outcrossing at, respectively, 46, 137 and 366 m from the pollen source. Discrepancies between distances of pollen flow may be due to differences in pollen donor plot size, which was 3–10 ha in the study of Timmons et al. (1996), while in the experiments of Scheffler et al. (1995) this was only 400 m^2. These results showed that care should be taken with predicting the probable performance of genetically modified oilseed rape under (semi-)standard agricultural conditions based on extrapolating information obtained from small-scale release experiments.

The above-mentioned outcrossing frequencies are based on pollen dispersal within populations. Ellstrand & Marshall (1985), however, concluded based on paternity analysis of radish populations that sometimes up to 20% contamination from adjacent populations and till 1000 m occurred. These data led Klinger et al. (1991) as well as Ellstrand & Marshall (1985) to suggest that long-range transport of pollen cannot be ruled out. Thus, although reported outcrossing frequencies are low, gene dispersal cannot be prevented. Therefore, precautions should be practised concerning predictions of pollen spread in general and oilseed rape pollen spread in particular as for this crop both insects and wind are vectors which act supplementarily; especially when wind pollination is involved, pollen spread cannot be ruled out.

Propagation and seed survival. Areas of concern that were identified to be associated with the release of genetically modified oilseed rape are two-fold (Crawley et al. 1993). Genetically modified oilseed rape itself may become a weed and/or invade natural habitats or releasing genetically modified oilseed rape may enable sexual transfer of the inserted genes to neighbouring commercial or natural populations whose offspring may then become (more) weedy or invasive.

Significant differences were found in the distribution of weedy characteristics among weeds, 'normal plants' and crops (Baker 1965; Keeler 1989). For the average crop plant, such as oilseed rape, to become as 'weedy' as the average weed it would need to acquire five weedy traits, which means the simultaneous acquisition of at least five gene substitutions. Therefore, it can be concluded that the probability of joint occurrence of new alleles producing significantly weedy plants from oilseed rape is extremely low (10^{-25}; Keeler 1989), provided pleiotropic effects giving stress tolerance are absent.

Oilseed rape propagates through seeds, which, when mature, disperse by pod shattering. A silique can contain 10–30 seeds (Downey et al. 1980). After a stay for 10 years in the soil 10% of the seed is still able to germinate (Cramer 1987). Crop rotation of oilseed rape and cereals is recommended as oilseed volunteers can then be controlled more easily (Cramer 1987). Crawley et al. (1993) did not find a significant effect of depth

of burial of rape seed on its survival, while seed survival of charlock (*Sinapis arvensis* L.), a weedy relative of oilseed rape, significantly increased with deeper burial.

In an extensive ecological study Crawley *et al.* (1993) also found significant differences in seed survival on burial between conventional and transgenic oilseed rape, where transgenic lines were less invasive and less persistent compared to non-transgenic lines. Although there was substantial variation in seed survival, neither plant growth and seed production between sites tested, experimental treatments performed, nor introduction of kanamycin resistance or herbicide tolerance through genetic modification seems to increase the invasive potential of oilseed rape. Therefore, oilseed rape will extinct in all experimental treatments and all habitats studied. In competition-free gaps, however, a successful recruitment of oilseed rape from seed might be possible.

There are two types of oilseed rape, a winter and a summer type. In contrast to the latter, which does not show seed dormancy, the first type needs a period of cold before it will germinate. Before winter the plant forms a rosette and in the spring an elongated flower stem is formed and the plant starts flowering. Such dormancy is a characteristic that contributes to the weedy nature of oilseed rape (Keeler 1989).

The transgenic trait will also influence the establishment of transgenic oilseed rape. Drought tolerance or disease resistance are expected to give a fitness advantage enhancing plant performance in natural habitats. Herbicide tolerance will only give a selective advantage if the herbicide is widely applied. In addition, also genetic drift, migration and mutation will influence this process (Evenhuis & Zadoks 1991; Van Raamsdonk 1995).

Hybridization

According to Hoffman (1990), Evenhuis & Zadoks (1991) and Darmency (1994) biosafety analyses of gene transfer have to deal with: (a) emission, dispersal and deposition of pollen from transgenic plants, (b) stable integration of the transgene in the host genome and introgression of the transgene into other (wild) species, (c) stabilization and spread of the transgene in such species and (d) ecological effects of the transgene in the new host population.

Hybridization within Cruciferae. In the family of *Cruciferae* for several decades inter-specific and inter-generic crosses have been performed for different purposes. Since U (1935) gave a clear view of the relationships between different *Brassica* species, many genetic analyses within the tribe of *Brassiceae* and within the family of *Cruciferae* were carried out for better understanding general genetic mechanisms (Yarnell 1956; Röbbelen 1960, 1966; Heyn 1977; Clauss 1978). McNaughton & Ross (1978) reviewed the possibilities for forage crop improvement through inter-specific and inter-generic hybridization. In this respect the development of new crops such as × *Brassicoraphanus* (Oost 1984) or *Raphanobrassica*, resulting from sexual hybridization between *R. sativus* and *B. oleracea* or *B. rapa* (Karpechenko 1928; Dolstra 1982; Prakash & Tsunoda 1983), more specifically called Radicole (RRCC, McNaughton 1979) and Raparadish (AARR, Toxopeus 1985; Lange *et al.* 1989) should be mentioned.

Hybridization of transgenic oilseed rape and related non-oilseed Brassica, Sinapis *and* Raphanus *species.* The success of hybridization between crops and wild relatives depends on the relationship between species involved. Dale (1994) extensively described factors which determine the likelihood of hybrids between crop plants and related

species and their possible establishment in agricultural or natural habitats. In the framework of the EU-BAP (Biotechnology Action Program (BAP) 1990) and EU-BRIDGE (Biotechnology Research for Innovation, Development and Growth in Europe 1992, 1993) projects, hybridization of transgenic, herbicide-tolerant oilseed rape and several related species has been studied (Scheffler & Dale 1994). Lefol *et al.* (1991) obtained 2–3% *in vitro*-produced hybrids between transgenic oilseed rape and *B. adpressa*. Also in the field, using male sterile oilseed rape, hybrids with *B. adpressa* and *Raphanus raphanistrum* were detected (Chèvre *et al.* 1992; Eber *et al.* 1994; Baranger *et al.* 1995). Such hybrids show normal female fertility. Male fertility is reported to be 13% and 35% for the hybrids with *B. adpressa* and *R. raphanistrum*, respectively (Eber *et al.* 1994). Pollen fertility varied from 1% to 30%.

Kerlan *et al.* (1992) described reciprocal crosses between herbicide-tolerant oilseed rape and five related species: *B. oleracea* L. var. *acephala*, *B. oleracea* L. var. *capitata*, *B. nigra* L. Koch, *B. adpressa* L., *Raphanus raphanistrum* and *Sinapis arvensis* L. The last three commonly occur in oilseed rape fields in France and were locally collected. All the inter-specific combinations tested were able to produce hybrid plants, but only when fertilized ovaries were established in *in vitro* culture. When rapeseed was used as a female parent more hybrid plants were obtained. Probably this can be explained by the higher chromosome number in oilseed rape, which was also found to influence hybridization capacity in other studies concerning reciprocal differences in yield of hybrid embryos (Mohapatra & Bajaj 1987; Quazi 1988). These observations show severe limitation in gene dispersal due to hybridization barriers. In contrast to the hybrids formed spontaneously, the *B. adpressa* and *R. raphanistrum* hybrids obtained by embryo rescue were mostly sterile (Eber *et al.* 1994). Also the other hybrids were male sterile or poorly fertile, except for two amphidiploid *B. napus* × *B. oleracea* plants, which showed a fertility comparable to oilseed rape. Such a reduced male fertility diminishes the possibility for gene dispersal.

Gene introgression after sexual hybridization depends on the percentage of chromosome pairing. The higher this percentage is, the higher the opportunity that a (trans)gene introgresses into the genomes of the wild relative. Therefore, Kerlan *et al.* (1993) studied the meiotic behaviour of the hybrids between herbicide-tolerant oilseed rape and the five related species earlier mentioned together with the physical presence and expression of the Basta® tolerance, bar gene. Most of the 75 hybrids studied had a triploid structure (ACX). Comparing the percentage chromosome pairing in the hybrids with that of haploid oilseed rape allosyndesis between rapeseed AC genomes and the genomes of related species occurred. The presence of multivalent association in all hybrids also indicated the possibility for recombination. Also a good correlation between presence of the bar gene and herbicide tolerance, providing the T-DNA was inserted as a single locus was observed. If the T-DNA was present at three loci, two plants having the bar gene were nevertheless found to be Basta® susceptible. This might be explained by suppression of gene expression through a position effect (De Block *et al.* 1989) or through DNA methylation followed by gene inactivation (Matzke *et al.* 1989; Hobbs *et al.* 1990; Linn *et al.* 1990). Other explanations might be partial complementation caused by an insufficient transgene expression, cosuppression (Flipse 1995) or (anti)sense inhibition (Jorgensen 1990; Grierson *et al.* 1991; Mol *et al.* 1994; Flipse 1995).

Based on the study of the occurrence and the cytogenetical characterization of inter-specific hybrids (Kerlan *et al.* 1992, 1993), the five related *Brassica* species were ranked by decreasing ecological impact: *B. oleracea*, *R. raphanistrum*, *B. adpressa*,

S. arvensis and *B. nigra*. Their results showed that gene transfer would not occur to the weedy relatives *B. nigra and S. arvensis* due to natural cross barriers, which is in agreement to that found by Downey & Bing (1990) and Bing *et al.* (1991, 1995). Between *B. rapa* and *B. nigra* gene transfer was shown to be possible, while gene transfer between *B. rapa* and *S. arvensis* was at the most difficult (Bing *et al.* 1996). Hybridization of oilseed rape and radish (*R. sativus*) was shown by Metz *et al.* (1995). However, this will not have an ecological impact. Although herbicide tolerance could be transferred from transgenic oilseed rape to the hybrid, hybridization was only possible under special laboratory conditions and, in addition, the hybrid plants were almost completely sterile.

Hybridization among (transgenic) oilseed Brassicas (B. napus, B. rapa *and* B. juncea*)*. *B. rapa*, *B. napus* and *B. juncea* are commercially grown oilseed species. The last two accommodate the *B. rapa* AA genome.

Hybridization of B. juncea *and* B. napus. *B. juncea*, cultivated in Asia, the United States and Canada for oil and mustard production, is found as a weed or ruderal in Denmark and Sweden (Frello *et al.* 1995). In Southern Europe it is naturalized (Heywood & Akeroyd 1993).

Inter-specific hybrids of *B. napus* and *B. juncea* are easy to obtain in controlled crosses with *B. juncea* as female parent while spontaneous hybridization is also observed (Bing *et al.* 1991; Frello *et al.* 1995). On the basis of RAPD analysis, a relatively high homology between the A-, B- and C-genome was found, making recombination between these chromosomes feasible (Quiros *et al.* 1991, 1994). Such introgression from oilseed rape into the genome of *B. juncea* has been reported (Frello *et al.* 1995), while hybridization between *B. juncea* and *B. rapa* has also been reported (Anand *et al.* 1985; Banga 1986). These hybridizations are not relevant for The Netherlands because *B. juncea* is not cultivated and very seldomly occurs in nature under Dutch circumstances.

Hybridization of B. rapa *and* B. napus. Turnip is an annual or biennial herb, cultivated on a modest scale. It is very frequently found on open waysides, disturbed ground and other unnatural habitats. (Sub)spontaneous populations are found in the wild, which might be regarded as wild relatives of oilseed rape. Also, many records of the escape of *B. napus* can be traced back concerning *B. rapa*.

De Vries *et al.* (1992) have made so-called botanical files for 42 species of cultivated plants grown in The Netherlands using a D_{pdf} code, consisting of three dispersal codes with six indices each. D_p gives an indication for gene dispersal by pollen, D_d for gene dispersal by seeds and diaspores and D_f for the frequency of the plants in the wild. The numerical code is a measure for the possible ecological effects of the cultivated plant on the wild flora of The Netherlands (Frietema De Vries 1996). Oilseed rape obtained a D_{pdf} code of 2.2.4, indicating that a medium ecological effect can be expected on the Dutch flora (Frietema De Vries 1996). Turnip (*B. rapa*) obtained a D_{pdf} code of 5.5.4, which indicates the expectation of a substantial and widespread ecological effect on the flora of The Netherlands. Under Dutch circumstances oilseed rape and turnip flower simultaneously from April to August (Van der Meijden 1990) and hybridization of *B. rapa* and *B. napus* has been reported to occur occasionally (De Vries *et al.* 1992).

Reports on the crossability between oilseed rape and *B. rapa* are nevertheless controversial (Jørgensen & Andersen 1994). In breeding programmes of oilseed rape, crosses with *B. rapa* were performed (Gowers 1982). Natural crosses between these species are thought to be either difficult and not likely to happen (Downey *et al.* 1980) or common in nature, which was exemplified by spontaneous hybridization in agricultural fields (Bing *et al.* 1991; Jørgensen & Andersen 1994). In Denmark, *B. rapa* is a

common weed in cultivated areas, mostly in oilseed rape fields (Jørgensen & Andersen 1994). The hybrid plants identified produced a small amount of viable seeds after open pollination, which indicated that these hybrids might survive the next generation.

The possibility for gene transfer from *B. napus* to *B. rapa* under natural circumstances will be less than observed under pollination conditions in field crossing blocks, because *B. rapa* flowers 1–2 weeks earlier than *B. napus* and because inter-specific hybridization is more successful when *B. rapa* is used as male parent (Downey & Rakow 1987; Bing *et al.* 1991; Bing 1991). It is in contradiction, however, to the results of Palmer (1962) who obtained, after open pollinations with an excess of pollen, on turnip 88% hybrid plants and on oilseed rape only 11% hybrid plants.

Hybrids had quantitatively a good pollen production, but showed reduced fertility (Beversdorf *et al.* 1980; Bing 1991). After staining, viability of the pollen was found to be about 60% (McNaughton 1973; MacKay 1977). The pollen fertility of the hybrid plants obtained from seeds harvested on *B. rapa* ranged from 21% to 86% in different experimental designs (Jørgensen & Andersen 1994). If plants were placed in a 1:1 mixture of *B. rapa* and oilseed rape, hybrids obtained from seeds harvested on oilseed rape plants had 41% (16–65%) pollen fertility. In contrast, Röbbelen (1966) observed in the cross *B. rapa* × *B. napus* complete sterility, which was suggested to be the result of aberrant embryo development.

Scheffler & Dale (1994) have reviewed the opportunities for hybridization between oilseed rape and related species. They also reported successful selfings and backcrosses of hybrids between turnip and oilseed rape. According to U (1935), McNaughton (1973) and Beversdorf *et al.* (1980) most such hybrids possessed 29 chromosomes. In the metaphase of the meiose 10 bivalents (the A genomes) and nine univalents (the C genome) were observed (U 1935; MacKay 1973; McNaughton 1973; Rousselle & Eber 1983). Apparently, the turnip chromosomes paired completely resulting in vital gametes.

Introgression is reported from *B. rapa* into *B. napus* (MacKay 1977; Goring *et al.* 1992). MacKay (1977) introgressed S-alleles from turnip and Goring *et al.* (1992) described the introgression of an S-locus glycoprotein cDNA. Because only oilseed rape was available with the desired cytoplasms, introgression of cold tolerance and black-rot resistance from *B. napus* into *B. rapa* (Pak choi) and *B. rapa* (Chinese cabbage) was accomplished (Guo *et al.* 1990; Heath *et al.* 1994).

We backcrossed hybrids of *B. rapa* and transgenic herbicide-tolerant oilseed rape to *B. rapa* (Metz 1995), while Mikkelsen *et al.* (1996) performed the reciprocal backcross. In the BC_1 and BC_2 generations herbicide tolerance was detected, which indicates that introgression of a transgene into *B. rapa* seems possible (Mikkelsen *et al.* 1996; Metz *et al.* 1997). These results show that if the natural conditions are as optimal as in the study of Mikkelsen *et al.* where inter-specific hybrids were grown in small plots together with *B. rapa* or as our experiment conditions where pollen is put in excess on the stigma of the receptor plant, a transgene can be transferred to *B. rapa*. This might confer a fitness advantage to *B. rapa* under selective conditions. However, it is difficult to conceive of a situation in which genetic modification for herbicide tolerance will influence the fitness of a plant in the absence of the herbicide (Gliddon 1994).

Introgression and gene transfer to *B. rapa* might be limited by introduction of a transgene into the C-genome of *B. napus*. It is expected that the transgene will probably be present in a lower than expected percentage of the plants after 2–3 generations of backcrossing with the wild relative *B. rapa*. However, in general, the occurrence of fertile, transgenic *B. rapa*-like plants after hybridization and two generations of

backcrossing suggests possible gene dispersal from oilseed rape to its weedy relative *B. rapa.*

Impact of transgene features on biosafety of transgenic oilseed rape
It can be concluded that complete containment of transgenic oilseed rape is not possible. In the case of hybrids of *B. rapa* and oilseed rape, studies on the stability of transgene expression over generations and in different genetic backgrounds are relevant. Such studies can show the possible impact of transgene action and stability in these hybrids.

In general, there will be a shift from the question of possible transgene escape to the question of the ecological and toxicological impact of the introduced genes (Timmons *et al.* 1996). By order of the Dutch Ministries of Economic Affairs and Housing, Spatial Planning and the Environment a series of literature reports was written about the so-called transgene-centred evaluation of genetically engineered plants in addition to that of kanamycin (Nap *et al.* 1992). The ecological and toxicological biosafety aspects of the phosphinothricin tolerance gene (Nap & Metz 1996) and the glyphosate tolerance gene (Nap *et al.* 1996) have been evaluated until now and more transgene-centred evaluations are in progress. Such a transgene-centred approach may prove the more useful in the near future (Metz & Nap, 1997, this issue).

CONCLUDING REMARKS

It is expected that transgenic oilseed rape will be at the forefront of commercialization of transgenic crops. Therefore, oilseed rape is a good model crop for biosafety studies. The taxonomy and cytogenetics of the family of *Cruciferae* give rise to ample possibilities for inter-specific and inter-generic hybridization, either with or without embryo-rescue techniques. Pollen is thought to be the main factor through which transgenes may spread. Vectors for pollination of oilseed rape are both insects and wind.

Monitoring pollen movements from (semi) commercial oilseed rape fields showed that extrapolating information obtained from small-scale release experiments must be done carefully. Although reported outcrossing frequencies were low, pollen spread and gene dispersal from transgenic oilseed rape to its (wild) relatives cannot be prevented.

Studies on reciprocal crosses between transgenic oilseed rape and a number of related species showed that all inter-specific and inter-generic combinations tested are able to produce hybrid plants, but in most cases only after elaborate *in vitro* culture. However, for some related species spontaneous hybridization has been reported under field conditions. Spontaneous hybridization among the oilseed Brassicas (*B. rapa, B. juncea* and *B. napus*) has been determined in agricultural fields. The occurrence of fertile, transgenic *B. rapa*-like plants after hybridization and two generations of backcrossing suggests gene dispersal from oilseed rape to its weedy relative *B. rapa* and introgression of oilseed rape genes in *B. rapa* is possible. Such gene dispersal and introgression might be limited by inserting the transgene in the C-genome. Studies on the stability of transgene expression over generations and in different genetic backgrounds can show the real impact in time of transgene action in hybrids of *B. rapa* and oilseed rape.

Because complete containment of transgenic oilseed rape is not possible, attention should now focus on the ecological and toxicological impact of the introduced genes.

Such transgene-centred ecological and toxicological evaluation, irrespective of the genetically engineered plant species, may prove useful in the near future.

REFERENCES

Anand, I.J., Mishra, P.K. & Rawat, D.S. (1985): Mechanism of male sterility in *Brassica juncea* I. Manifestation of sterility and fertility restoration. *Cruciferae Newsl.* **10**: 44–46.

Animal and Plant Health Service (APHIS)/US Department of Agriculture (USDA) (1994): Response to Petition 94-090-01p from Calgene Inc. regarding *Laurate canola.*

Appelqvist, L.Å. (1972): Historical background. In: Appelqvist, L.Å. & Ohlson, R. (eds.): *Rapeseed; cultivation, composition, processing and utilization,* pp. 1–8, Elsevier, Amsterdam, London, New York.

Baker, H.G. (1965): Characteristics and modes of origin of weeds. In: Baker, H.G. & Stebbins, G.L. (eds.): *The Genetics of Colonizing Species,* p. 147, Academic Press, New York.

Banga, S.S. (1986): Hybrid pollen-aided induction of matromorphy in *Brassica. Z. Pflanzenzücht.* **96**: 86–89.

BAP (1990): Study of gene dispersal from plants produced by recombinant DNA technology. In: *Proceedings of the Final Sectorial EC BAP Meeting,* Padua, Italy, 17–21 December 1990.

Baranger, A., Chèvre, A.M., Eber, F. & Renard, M. (1995): Effect of oilseed rape genotype on the spontaneous hybridization rate with a weedy species: an assessment of transgene dispersal. *Theor. Appl. Genet.* **91**: 956–963.

Becker, H.C., Damgaard, C. & Karlsson, B. (1992): Environmental variation for outcrossing rate in rapeseed (*Brassica napus*). *Theor. Appl. Genet.* **84**: 303–306.

Beversdorf, W.D., Weiss-Lerman, J., Erickson, L.R. & Souza Machado, V. (1980): Transfer of cytoplasmically-inherited triazine resistance from bird's rape to cultivated oilseed rape (*Brassica capestris* and *B. napus*). *Can. J. Genet. Cytol.* **22**: 167–172.

Bing, D.J., Downey, R.K. & Rakow, G.F.W. (1996): Assessment of transgene escape from *Brassica rapa* (*B. campestris*) into *B. nigra* or *Sinapis arvensis. Plant Breeding* **115**: 1–4.

Bing, D.J., Downey, R.K. & Rakow, G.F.W. (1995): An evaluation of the potential intergeneric gene transfer between *B. napus* and *S. arvensis. Plant Breeding* **114**: 481–484.

Bing, D.J., Downey, R.K. & Rakow, G.F.W. (1991): Potential of gene transfer among oilseed *Brassica* and their weedy relatives. In: *Proceedings of the 8th*

International Rapeseed congress, Saskatoon, Canada, pp. 1022–1027.

Bing, D.J. (1991): *Potential of gene transfer among oilseed* Brassica *and their weedy relatives.* MSc Thesis, University of Saskatchewan, Saskatoon, Canada.

BRIDGE (1993): Safety assessment of the deliberate release of two model transgenic crop plants, oilseed rape and sugar beet. In: *Proceedings of the Final Sectorial EC BRIDGE Meeting on Biosafety,* Granada, Spain, 24–27 October 1993.

BRIDGE (1992): Safety assessment of the deliberate release of two model transgenic crop plants, oilseed rape and sugar beef. In: *Proceedings of the EC BRIDGE Meeting on Biosafety,* Wageningen, The Netherlands, 6–9 December 1992.

Brown, A.D. & Dyer, A.F. (1991): Effects of low temperature storage on the pollen of *Brassica-campestris, B. oleracea* and *B. napus. Euphytica* **51**: 215–218.

Brunel, E., Mesquida, J., Renard, M. & Tanguy, X. (1994): Repartition de l'entomofaune pollinsatrice sur des fleurs de colza (*Brassica napus* L.) et de navette (*Brassica campestris* L.): incidence du caractère apetale de la navette. *Apidologie* **25**: 12–20.

Chèvre, A.M., Renard, M., Eber, F., Vallee, P., Dechamps, M. & Kerlan, M.C. (1992): Study of spontaneous hybridization between male-sterile rapeseed and weeds. In: *Proceedings of the XIIIth Eucarpia Congress on Reproductive Biology and Plant Breeding,* Angers, France, 6–11 July 1992.

Chyi, Y.-S., Hoenecke, M.E. & Sernyk, J.L. (1992): A genetic linkage map of restriction length polymorphism loci for *Brassica rapa* (syn. *campestris*). *Genome* **35**: 746–757.

Clapham, A.R., Tutin, T.G. & Warburg, E.F. (1958): *Flora of the British Isles,* p. 1591, Cambridge University Press, Cambridge.

Clauss, E. (1978): Allohexaploide Gattungsbastarde vom Typ *Brassico-Raphanobrassica. Archiv für Züchtungsforsch. Berl.* **8**: 297–302.

Cramer, N. (1987): Durchwuchs im 00-Raps: das Qualitätsproblem Nr 1. *Top Agrar.* **7**: 44–49.

Crawley, M.J., Hails, R.S., Rees, M., Kohn, D. & Buxton, J. (1993): Ecology of transgenic oilseed rape in natural habitats. *Nature* **363**: 620–623.

Dale, P.J. (1994): The impact of hybrids between genetically modified crop plants and their related species: general considerations. *Mol. Ecol.* **3**: 31–36.

Dale, P.J. (1993): The release of transgenic plants into agriculture (review). *J. Agric. Sci. Cambridge* **120**: 1–5.

Dale, P.J. & Irwin, J.A. (1995): The release of transgenic plants from containment, and the move towards their widespread use in agriculture. *Euphytica* **85**: 425–431.

Dale, P.J., Irwin, J.A. & Scheffler, J.A. (1993): The experimental and commercial release of transgenic crop plants. *Plant Breeding* **111**: 1–22.

Darmency, H. (1994): The impact of hybrids between genetically modified crop plants and their related species: introgression and weediness. *Mol. Ecol.* **3**: 37–40.

De Block, M., Debrouwer, D. & Tenning, P. (1989): Transformation of *Brassica napus* and *Brassica oleracea* using *Agrobacterium tumefaciens* and the expression of the bar and neo genes in transgenic plants. *Plant Physiol.* **91**: 694–701.

De Greef, W. (1990): The release of transgenic plants into the environment: a review of the BAP projects. In: Economidis, I. (ed.): *Biotechnology R&D in the EC (Biotechnology Action Program BAP), Vol. I Catalogue of BAP achievements on risk assessment for the period 1985–1990*, pp. 19–22, Elsevier, Amsterdam.

Demeke, T., Adams, R.P. & Chibbar, R. (1992): Potential taxonomic use of random amplified polymorphic DNA (RAPD): a case study in *Brassica*. *Theor. Appl. Genet.* **84**: 990–994.

Den Nijs, A.P.M. (1989): Pollen als vector voor transgenen. *Prophyta* **43**: 225–227 [in Dutch].

De Vries, F.T., Van der Meijden, R. & Brandenburg, W.A. (1992): Botanical files: a study of the real chances for spontaneous gene flow from cultivated plant to the wild flora of the Netherlands. *Gorteria* Suppl. 1.

De Wet, J.M.J. & Harlan, J.R. (1975): Weeds and domesticates: evolution in the man-made habitat. *Econ. Bot.* **29**: 99–107.

Dolstra, O. (1982): *Synthesis and fertility of × Brassicoraphanus and ways of transferring Raphanus characters to Brassica*, PhD Thesis, LH Wageningen. Pudoc Centre for Agricultural Publishing and Documentation.

Downey, R.K. & Bing, D.J. (1990): Biosafety of transgenic oilseed crucifers. *Workshop on safeguards for planned introductions of transgenic oilseed crucifers*, Ithaca, NY, 9 October 1990. USDA-Aphis, Hyattsville, Maryland.

Downey, R.K. & Rakow, G.F.W. (1987): Rapeseed and mustard. In: Feyr, W. (ed.): *Principles of Cultivar Development, Vol. 2*, pp. 437–486, Macmillan, New York.

Downey, R.K., Klassen, A.J. & Stringham, G.R. (1980): Rapeseed and mustard. In: Fehr, W.R. & Hadley, H.H. (eds.): *Hybridization of Crop Plants*, pp. 495–509, American Society of Agronomy and Crop Science Society of America, Publishers Madison, Wisconsin.

Eber, F., Chèvre, A.M., Baranger, A., Vallée, P., Tanguy, X. & Renard, M. (1994): Spontaneous hybridization between a male-sterile oilseed rape and two weeds. *Theor. Appl. Genet.* **88**: 362–368.

Ellstrand, N.C. & Marshall, D.L. (1985): Interpopulation gene flow by pollen in wild radish, *Raphanus sativus*. *Amer. Nat.* **126**: 606–616.

Erickson, L.R., Straus, N.A. & Beversdorf, W.D. (1983): Restriction patterns reveal origins of chloroplast genomes in *Brassica* amphiploids. *Theor. Appl. Genet.* **65**: 201–206.

Evenhuis, A. & Zadoks, J.C. (1991): Possible hazards to wild plants of growing transgenic plants. A contribution to risk analysis. *Euphytica* **55**: 81–84.

Flipse, E. (1995): *The amylose-free potato mutant as a model plant to study gene expression and gene silencing*, PhD Thesis, Agricultural University Wageningen, The Netherlands.

Frello, S., Hansen, K.R., Jensen, J. & Jørgensen, R.B. (1995): Inheritance of rapeseed (*Brassica napus*)-specific RAPD markers and a transgene in the cross *B. juncea* × (*B. juncea* × *B. napus*). *Theor. Appl. Genet.* **91**: 236–241.

Frietema De Vries, F.T. (1996): *Cultivated plants and the wild flora; effect analysis by dispersal codes*, PhD Thesis, State University Leiden, The Netherlands.

Gliddon, C. (1994): Herbicide resistance in common UK crops: considerations of safety at different scales both for the natural and the agricultural environment. In: *Report of the Workshop 'Safety considerations of herbicide-resistant plants to be placed on the European Market'*, Brussels, Belgium, 26 January 1994.

Goring, D.R., Banks, P., Beversdorf, W.C. & Rothstein, S.J. (1992): Use of the polymerase chain reaction to isolate an S-locus glycoprotein cDNA introgressed from *Brassica campestris* into *B. napus* ssp. *oleifera*. *Mol. Gen. Genet.* **234**: 185–192.

Gowers, S. (1982): The transfer of characters from *Brassica campestris* L. to *Brassica napus* L.: production of clubroot-resistant oilseed rape (*B. napus* ssp. *oleifera*). *Euphytica* **31**: 971–976.

Grierson, D., Fray, R.G., Hamilton, A.J., Smith, C.J.S. & Watson, C.F. (1991): Does co-suppression of sense genes in transgenic plants involve antisense RNA? *Trends Biotech.* **9**: 122–123.

Guo, Z.H., Dickson, M.H. & Hunter, J.E. (1990): *Brassica napus* sources of resistance to black rot in crucifers and inheritance of resistance. In: *Proceedings of the 6th Crucifer Genetics Workshop*, Ithaca (NY), US, 7–9 October 1990.

Heath, D.W., Earle, E.D. & Dickson, M.H. (1994): Introgressing cold-tolerant Ogura cytoplasms from rapeseed into Pak choi and Chinese cabbage. *HortSci.* **29**: 202–203.

Heyn, F.W. (1977): Analysis of unreduced gametes in the *Brassiceae* by crosses between species and ploidy levels. *Z. Pflanzenzücht.* **78**: 13–30.

Heywood, V.H. & Akeroyd, J.R. (1993): *Brassica* L. In: Tutin, T.G., Burges, N.A., Chater, A.O., Edmondson, J.R., Heywood, V.H., Moore, D.M., Valentine D.H., Walters, S.M. & Webb, D.A. (eds.): *Flora Europaea*, pp. 405–409, Cambridge University Press, Cambridge.

Heywood, V.H., Moore, D.M., Richardson, I.B.K. & Stearn, W.T. (1993): *Flowering Plants of the World* (2nd edn), p. 335, Batsford Ltd, London.

Hobbs, S.L.A., Kpodar, P. & DeLong, C.M.O. (1990): The effects of T DNA copy number, position and methylation on reporter gene expression in tobacco transformants. *Plant Mol. Biol.* **15**: 851–864.

Hoffman, C.A. (1990): Ecological risks of genetic engineering of crop plants. *BioScience* **40**: 434–437.

Hosaka, K., Kianian, S.F., McGrath, J.M. & Quiros, C.F. (1990): Development and chromosomal localization of genome-specific DNA markers of *Brassica* and the evolution of amphidiploids and n=9 diploid species. *Genome* **33**: 131–142.

Johnston, T.D. & Jones, D.I.H. (1966): Variations in thiocyanate content of kale varieties. *J. Sci. Food Agric.* **17**: 70–71.

Jorgensen, R. (1990): Altered gene expression in plants due to *trans* interactions between homologous genes. *Trends Biotech.* **8**: 340–344.

Jørgensen, R.B. & Andersen, B. (1994): Spontaneous hybridization between oilseed rape (*Brassica napus*) and weedy *B. campestris* (*Brassicaceae*): a risk of growing genetically modified oilseed rape. *Amer. J. Bot.* **81**: 1620–1626.

Kapteijns, A.J.A.M. (1993): Risk assessment of genetically modified crops. Potential of four arable crops to hybridize with the wild flora. *Euphytica* **66**: 145–149.

Karpechenko, G.D. (1928): Polyploid hybrids of *Raphanus sativus* L. × *Brassica oleracea* L. *Z. indukt. Abstamm. Vererb. Lehre* **48**: 1–85.

Keeler, K.H. (1989): Can genetically engineered crops become weeds? *Bio/Technology* **7**: 1134–1139.

Kerlan, M.C., Chèvre, A.M. & Eber, F. (1993): Interspecific hybrids between a transgenic rapeseed (*Brassica napus*) and related species: cytogenetical characterization and detection of the transgene. *Genome* **36**: 1099–1106.

Kerlan, M.C., Chèvre, A.M., Eber, F., Baranger, A. & Renard, M. (1992): Risk assessment of outcross-ing of transgenic rapeseed to related species: I Interspecific hybrid production under optimal conditions with emphasis on pollination and fertilization. *Euphytica* **62**: 145–153.

Klinger, T., Elam, D.R. & Ellstrand, N.C. (1991): Radish as a model system for the study of engineered gene escape rates via crop–weed mating. *Conserv. Biol.* **5**: 531–535.

Landry, B.S., Hubert, N., Etoh, T., Harada, J.J. & Lincoln, S.E. (1991): A genetic map for *Brassica napus* based on restriction fragment length polymorphisms detected with expressed DNA sequences. *Genome* **34**: 543–552.

Lange, W., Toxopeus, H., Lubberts, J.H., Dolstra, O. & Harrewijn, J.L. (1989): The development of Raparadish (× *Brassicoraphanus* 2n=38), a new crop in agriculture. *Euphytica* **40**: 1–14.

Lefol, E., Danielou, V., Darmency, H., Kerlan, M.C., Vallee, P., Chèvre, A.M., Renard, M. & Reboud, X. (1991): Escape of engineered genes from rapeseed to wild *Brassiceae*. *Brighton Crop Protection Conference—Weeds, 1991, 8A-7:* 1049–1056.

Lelivelt, C.L.C. (1993): *Introduction of beet cyst nematode resistance from* Sinapis alba *L. and* Raphanus sativus *L. into* Brassica napus *L. (oil-seed rape) through sexual and somatic hybridization*, PhD Thesis, Agricultural University Wageningen, The Netherlands.

Linn, F., Heidmann, I., Saedler, H. & Meyer, P. (1990): Epigenetic changes in the expression of the maize A1 gene in Petunia hybrida: role of number of integrated gene copies and state of methylation. *Mol. Gen. Genet.* **222**: 329–336.

Linnaeus, C. (1745): Öländska och Gothländska Resa, Stockholm och Uppsala [in Swedish].

Lydiate, D., Sharpe, A., Lagercrantz, U. & Parkin, I. (1993): Mapping the *Brassica* genome. *Outlook Agric.* **22**: 85–92.

Lydiate, D. (1996): Genome organisation and gene transfer in cruciferous plants. In: *Final program & abstracts guide Plant Genome V Conference*, 12, San Diego (CA), US, 14–18 January 1996.

MacKay, G.R. (1977): The introgression of S alleles into forage rape, *Brassica napus* L. from turnip, *Brassica campestris* L. ssp. *rapifera*. *Euphytica* **26**: 511–519.

MacKay, G.R. (1973): Interspecific hybrids between forage rape (*Brassica napus* L.) and turnip (*Brassica campestris* L. ssp. *rapifera*) as alternatives to forage rape. 1. An exploratory study with single pair crosses. *Euphytica* **22**: 495–499.

Maessen, G.D.F. (1997): Genomic stability and stability of expression in genetically modified plants. *Acta Bot. Neerl.* **46**: 3–24.

Matzke, M.A., Primig, M., Trnovsky, J. & Matzke, A.J.M. (1989): Reversible methylation and

inactivation of marker genes in sequentially transformed tobacco plants. *EMBO J.* **8**: 643–649.

McNaughton, I.H. (1979): The current position and problems in the breeding of *Raphanobrassica* (Radicole) as a fodder crop. In: Marrewijk, N.P.A. van & Toxopeus, H. (eds.): *Proceedings of the Eucarpia Conference Cruciferae 1979*, pp. 22–28, SVP & RIVRO, Wageningen.

McNaughton, I. H. (1973): *Brassica napocampestris* L. (2n=58). 1. Synthesis, cytology, fertility and general considerations. *Euphytica* **22**: 301–309.

McNaughton, I.H. & Ross, C.L. (1978): Inter-specific and inter-generic hybridization in the *Brassicae* with special emphasis on the improvement of forage crops. *Ann. Rep. Scottish Plant Breeding Station*: 75–110.

Metz, P.L.J. (1995): Analyse van de overdracht van genen van transgene koolgewassen. Voortgangsrapport EZ project IOP-b 39401, January 1995 [in Dutch].

Metz, P.L.J. & Nap, J.P. (1997): A transgene-centred approach to the biosafety of transgenic crops: overview of selection and reporter genes. *Acta Bot. Neerl.* (this issue).

Metz, P.L.J., Nap, J.P. & Stiekema, W.J. (1995): Hybridization of radish (*Raphanus sativus* L.) and oilseed rape (*Brassica napus* L.) through a flower-culture method. *Euphytica* **83**: 159–168.

Metz, P.L.J., Jacobsen, E., Nap, J.P., Pereira, A. & Stiekema, W.J. (1997): The impact on biosafety of the phosphinothricin tolerance transgene in inter-specific *B. rapa* × *B. napus* hybrids and their successive backcrosses. *Theor. Appl. Genet.* (in press).

Mikkelsen, T.R., Andersen, B. & Jørgensen, R.B. (1996): The risks of crop transgene spread. *Nature* **380**: 31.

Mohammad, A. (1935): Pollination studies in toria (*Brassica napus* L. var. dichotoma Prain.) and sarson (*Brassica campestris* L. var. sarson, Prain.). *Ind. J. Agric. Sci.* **5**: 125–154.

Mohapatra, D. & Bajaj, Y.P.S. (1987): Interspecific hybridization in *Brassica juncea* × *Brassica hirta* using embryo rescue. *Euphytica* **36**: 321–326.

Mol, J.N.M., van Blokland, R., de Lange, P., Stam, M. & Kooter, J.M. (1994): Post-transcriptional inhibition of gene expression: sense and antisense genes. In *Paszkowski, J. (ed.): Homologous recombinations and gene silencing in plants*, pp. 309–314, Kluwer Academic, The Netherlands.

Morris, W.F., Kareiva, P.M. & Raymer, P.L. (1994): Do barren zones and pollen traps reduce gene escape from transgenic crops? *Ecol. Appl.* **4**: 157–164.

Nap, J.-P., Bijvoet, J. & Stiekema, W.J. (1992): Biosafety of kanamycin-resistant transgenic plants. *Transgen. Res.* **1**: 239–249.

Nap, J.-P. & Metz, P.L.J. (1996): A transgene-centered evaluation of transgenic plants. Part 2. Biosafety of transgenic phosphinothricin-tolerant plants. CPRO-DLO Report (CCRO-VROM project).

Nap, J.-P., Metz, P.L.J. & Stiekema, W.J. (1996): A transgene-centered evaluation of transgenic plants. Part 3. Biosafety of transgenic glyphosate-tolerant plants. CPRO-DLO Report (CCRO-VROM project).

Ohlson, R. (1972): Production of and trade in rapeseed. In: Appelqvist, L.Å. & Ohlson, R. (eds.): *Rapeseed; cultivation, composition, processing and utilization*, pp. 9–35, Elsevier, Amsterdam, London, New York.

Olsson, G. (1960): Species crosses within the genus *Brassica* II. Artificial *Brassica napus. Hereditas* **46**: 351–386.

Oost, E. (1984): × *Brassicoraphanus* Sageret or × *Raphanobrassica* Karpechenko? *Cruciferae Newsl.* **9**: 11–12.

Organization for Economic Cooperation and Development (OECD) (1993a): *Safety Considerations for Biotechnology: scale-up of crop plants*, OECD, Paris.

Organization for Economic Cooperation and Development (OECD) (1993b): *Safety Evaluation of Foods Derived by Modern Biotechnology; Concepts and principles*, OECD, Paris.

Organization for Economic Cooperation and Development (OECD) (1993c): *Field Releases of Transgenic Plants 1986–1992—an analysis*, OECD, Paris.

Palmer, J.D., Shields, C.R., Cohen, D.B. & Orton, T.J. (1983): Chloroplast DNA evolution and the origin of amphidiploid *Brassica* species. *Theor. Appl. Genet.* **65**: 181–189.

Palmer, T.P. (1962): Population structure, breeding system, inter-specific hybridization and allopolyploidy. *Heredity* **17**: 278–283.

Parkin, I.A.P., Sharpe, A.G., Keith, D.J. & Lydiate, D.J. (1995): Identification of the A and C genomes of amphidiploid *Brassica napus* (oilseed rape). *Genome* **38**: 1122–1131.

Pauk, J., Stefanov, I., Fekete, S., Bogre, L., Karsai, I., Fehér, A. & Dudits, D. (1995): A study of different (CaMV 35S and *MAS*) promoter activities and risk assessment of their field use in transgenic rapeseed plants. *Euphytica* **85**: 411–416.

Paul, E.M., Thompson, C. & Dunwell, J.M. (1995): Gene dispersal from genetically modified oil seed rape in the field. *Euphytica* **81**: 283–289.

Prakash, S. & Tsunoda, S. (1983): Cytogenetics in *Brassica*. In: Swaminathan, M.S., Gupta, P.K. & Sinha, U. (eds.): *Cytogenetics of Crop Plants*, pp. 482–513, Macmillan, Delhi.

Quazi, M.H. (1988): Interspecific hybrids between *Brassica napus* L. and *B. oleracea* L. developed by embryo culture. *Theor. Appl. Genet.* **75**: 309–318.

Quiros, C.F., Hu, J., This, P., Chevre, A.M. & Delseny, M.(1991): Development and chromosomal localization of genome-specific markers by polymerase chain reaction in *Brassica. Theor. Appl. Genet.* **82**: 627–632.

Quiros, C.F., Hu, J. & Truco, M.J. (1994): DNA-based marker maps of *Brassica*. In: Phillips, R.L. & Vasil, I.K. (eds.): *DNA-based markers in plants*, pp. 199–222, Kluwer Academic, The Netherlands.

Rakow, G. & Woods, D.L. (1987): Outcrossing in rape and mustard under Saskatchewan prairie conditions. *Can. J. Plant Sci.* **67**: 147–151.

Renard, M., Louter, J.H. & Duke, L.H. (1993): Oilseed rape. In: *Traditional Crop Breeding Practices: an historical review to serve as a baseline for assessing the role of modern biotechnology*, pp. 147–157, OECD, Paris.

Röbbelen, G. (1966): Beobachtungen bei interspezifischen *Brassica* Kreuzungen, insbesondere über die Enstehung matromorphen F1 Pflanzen. *Angewandte Bot.* **39**: 205–221.

Röbbelen, G. (1960): Beiträge zur Analyse des *Brassica* Genomes. *Chromosoma (Berl.)* **11**: 205–228.

Rouselle, P. & Eber, F. (1983): Crosement interspécifique entre quelques *Brassicae* et *Brassica napus* L. Analyse génomique des hybrides et perspectives d'obtention de systèmes d'androstérilité chez le colza. *Agronomie* **3**: 153–159.

Scheffler, J.A. & Dale, P.J. (1994): Opportunities for gene transfer from transgenic oilseed rape (*Brassica napus*) to related species. *Transgen. Res.* **3**: 263–278.

Scheffler, J.A., Parkinson, R. & Dale, P.J. (1995): Evaluating the effectiveness of isolation distances for field plots of oilseed rape (*Brassica napus*) using a herbicide-resistance transgene as selectable marker. *Plant Breeding* **114**: 317–321.

Scheffler, J.A., Parkinson, R. & Dale, P.J. (1993): Frequency and distance of pollen dispersal from transgenic oilseed rape (*Brassica napus*). *Transgen. Res.* **2**: 356–364.

Schiemann, E. (1932): Entstehung der Kulturpflanzen. In: Baur, E. & Hartmann, M. (eds.): *Handbuch der Vererbungswissenschaft Band III*, pp. 271–288, Verlag Gebrüder Borntraeger, Berlin.

Sharpe, A.G., Parkin, I.A.P., Keith, D.J. & Lydiate, D.J. (1995): Frequent nonreciprocal translocations in the amphidiploid genome of oilseed rape (*Brassica napus*). *Genome* **38**: 1112–1121.

Singh, D. (1958): *Rape and Mustard*, Indian Central Oilseed Committee, Bombay.

Slocum, M.K., Figdore, S.S., Kennard, W.C., Suzuki, J.Y. & Osborne, T.C. (1990): Linkage arrangement of restriction fragment length polymorphism loci in *Brassica oleracea. Theor. Appl. Genet.* **80**: 57–64.

Song, K.M., Suzuki, J.Y., Slocum, M.K., Williams, P.H. & Osborn, T.C. (1991): A linkage map of *Brassica rapa* (syn. *campestris*) based on nuclear restriction fragment length polymorphism loci. *Theor. Appl. Genet.* **82**: 296–304.

Song, K.M., Osborn, T.C. & Williams, P.H. (1990): *Brassica* taxonomy based on nuclear restriction fragment length polymorphisms (RFLPs). 3. Genome relationship in *Brassica* and related genera and origin of *B. oleracea* and *B. rapa* (syn. *campestris*). *Theor. Appl. Genet.* **79**: 497–506.

Stiekema, W.J. & Van Vloten-Doting, L. (1991): Application of transgenic crops. *ETC Course 'Introduction of genetically modified organisms into the environment: biosafety aspects'*, Wageningen, The Netherlands, 4–8 December.

Thormann, C.E., Ferreira, M.E., Camargo, L.E.A., Tivang, J.G. & Osborn, T.C. (1994): Comparison of RFLP and RAPD markers to estimating genetic relationships within and among cruciferous species. *Theor. Appl. Genet.* **88**: 973–980.

Timmons, A.M., Charters, Y.M., Crawford, J.W., Burn, D., Scott, S.E., Dubbels, S.J., Wilson, N.J., Robertson, A., O'Brien, E.T., Squire, G.R. & Wilkinson, M.J. (1996): Risks from transgenic crops. *Nature* **380**: 487.

Timmons, A.M., O'Brien, E.T., Charters, Y.M., Dubbels, S.J. & Wilkinson, M.J. (1995): Assessing the risks of wind pollination from fields of genetically modified *Brassica napus* ssp. *oleifera. Euphytica* **85**: 417–423.

Toxopeus, H. (1985): × *Brassicoraphanus* Sageret, cultivargroup Raparadish. *Cruciferae Newsl.* **10**: 13.

U N (1935): Genome analysis in *Brassica* with special reference to the experimental formation of *B. napus* and peculiar mode of fertilization. *Jap. J. Bot.* **7**: 389–452.

Van der Meijden, R. (1990): *Heukels Flora van Nederland*, 21ste druk, Wolters Noordhoff, Groningen [in Dutch].

Van Raamsdonk, L.W.D. (1995): The effect of domestication on plant evolution. *Acta Bot. neerl.* **44**: 421–438.

Van Raamsdonk, L.W.D. & Schouten, H.J. (1997): Gene flow and establishment of transgenes in natural plant populations. *Acta Bot. Neerl.* **46**: 69–84.

Verma, S.C. & Rees, H. (1974): Nuclear DNA and the evolution of allotetraploid *Brassicae. Heredity* **33**: 61–68.

Ward, M. (1994): EU plans to streamline GMO regulations. *Bio/Technology* **12**: 864.

Williams, I.H., Martin, A.P. & White, R.P. (1986): The pollination requirements of oil-seed rape (*Brassica napus* L.). *J. Agric. Sci. Cambridge* **106**: 27–30.

Williams, I.H. (1978): The pollination requirements of swede rape (*Brassica napus* L.) and of turnip rape (*Brassica campestris* L.). *J. Agric. Sci. Cambridge* **91**: 343–348.

Wodehouse, R.P. (1935): *Pollen Grains; their structure, identification and significance in science and medicine*, McGraw-Hill, New York.

Yarnell, S.H. (1956): Cytogenetics of the vegetable crops. 2. Crucifers. *Bot. Rev.* **22**: 81–166.

Zeven, A.C. (1975): The beginning of agriculture and sequence of plant domestication. In: Zeven, A.C. & Zhukovsky, P.M. (eds.): *Dictionary of Cultivated Plants and Centres of Diversity*, pp. 9–17, Pudoc, Wageningen.

Zeven, A.C. (1977): Domesticatie en evolutie van de cultuurplanten. *College syllabus Plant Breeding, Agricultural University, Wageningen* [in Dutch].