

Brassica, Oilseed Rape

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1. Taxonomy

1.1. General remarks

The Brassicaceae is a large plant family (338 genera and 3700 species) of major scientific and economic importance. Almost 100 years after the first taxonomic and systematic treatise on the family of the Brassicaceae (Hayek von, 1911) and subsequent contributions (Schulz, 1936) (Janchen, 1947) (Koch et al., 2003) we are now close to the first comprehensive and natural system regarding the mustard family. The increasing importance of *Arabidopsis* and *Brassica* as model organisms in Plant Sciences has greatly advanced systematics and taxonomy as well as evolutionary and developmental research on the entire family for two reasons: First, the most modern and recently developed molecular tools developed for the model plants were made available and have been applied successfully to wild relatives, and, second, it is important to prove these conclusions reached for the model plants, also in their wild relatives.

1.2. Phylogenetic relationships

1.2.1. *Phylogenetic relationships of Brassicaceae*

A most actual overview on Brassicaceae phylogeny and systematics is provided by (Al-Shehbaz et al., 2006) and a new tribal system is suggested reflecting phylogenetic relationships

A critical review of characters used in the systematics of the Brassicaceae is given, and aspects of the origin, classification, and generic delimitation of the family discussed. Molecular phylogenetic studies of the family were reviewed, and major clades identified. Based on molecular studies, especially from the *ndhF* chloroplast gene, and careful evaluation of morphology and generic circumscriptions, a new tribal alignment of the Brassicaceae is proposed. In all, 25 tribes are recognized, of which seven (*Aethionemeae*, *Boechereae*, *Descurainieae*, *Eutremeae*, *Halimolobeae*, *Noccaeeae*, and *Smelowskieae*) are described as new. For each tribe, the center(s) of distribution, morphology, and number of taxa are given. Of the 338 genera currently recognized in the Brassicaceae, about 260 genera (or about 77%) were either assigned or tentatively assigned to the 25 tribes.

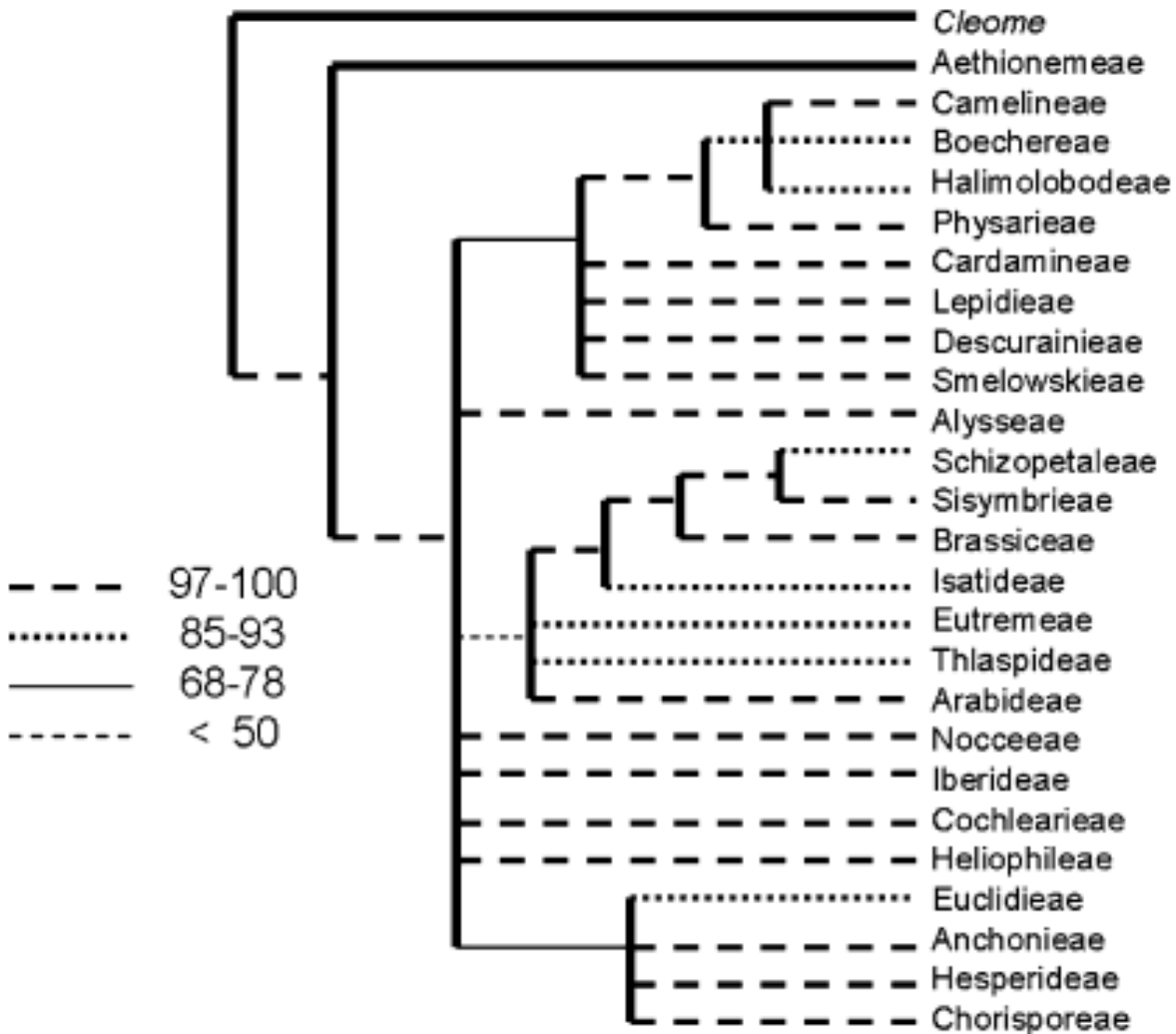


Fig. 1 Phylogenetic relationships among tribes of the Brassicaceae (modified from (Beilstein et al., 2006)).

1.2.2. Phylogenetic relationships within Brassicaceae

(Warwick & Black, 1991) studied the genetic relationship within Brassicaceae: Chloroplast DNA restriction sites for 20 endonucleases were mapped using cpDNA probes from *Brassica juncea* and site variation was surveyed in 33 diploid taxa of the Subtribe Brassicinae. A total of 419 mutations was observed, including both site (i.e., gain/ loss) and fragment length (i.e., insertions or deletions); 221 (53%) mutations showed variation at the interspecific level. Phylogenetic analysis indicated a clear division of the subtribe into two ancient evolutionary lineages. These were (I) the “Nigra” lineage: *Brassica nigra*, *B. fruticulosa*, *B. tournefortii*, *Sinapis pubescens*, *S. alba*, *S. flexuosa*, *S. arvensis*, *Coincya cheiranthos*, *Erucastrum canariense*, and *Hirschfeldia incana*, and (II) the “Rapa Oleracea” lineage: *Brassica rapa*, *B. oleracea* ssp. *oleracea* and ssp. *alboglabra*, *B. rupestris-villosa* complex (B.

rupestris, *B. drepanensis*, *B. macrocarpa*, *B. villosa*), *B. barrelieri*, *B. deflexa*, *B. oxyrhina*, *B. gravinae*, *Diplotaxis eruroides*, *D. tenuifolia*, *Eruca sativa*, *Raphanus raphanistrum*, *R. sativus*, and *Sinapis aucheri*. In the “Nigra” lineage, *Brassica nigra* was most closely related to the annual *Sinapis* species, *S. arvensis* and *S. alba*. In the “Rapa/Oleracea” lineage, the *Brassica rapa* and *B. oleracea* genomes formed distinct group whose closest relatives were the wild species of the *B. oleracea* ($n=9$) complex (i.e., *B. rupestris-villosa* complex). Species with $n=7$ chromosomes exist in both lineages. *Hirschfeldia incana* ($n = 7$), in the “Nigra” lineage, was most closely related to *Sinapis pubescens*. In the “Rapa/Oleracea” lineage three taxa with $n = 7$ - *B. deflexa*, *D. eruroides*, and *S. aucheri* - were closely related, advanced in the lineage, and were the closest apparent relatives (particularly *D. eruroides*) to *B. rapa*, *B. oleracea*, and its wild relatives. Levels of genetic divergence suggested by the cpDNA data were consistent with cytodeme recognition in the subtribe, but provided evidence for inconsistencies in the current generic delimitations based on morphology. Very low levels of genetic divergence were evident among taxa/accessions within a cytodeme. *Raphanus* was closely related to the *Brassica rapa* and *B. oleracea* genomes and clearly belongs in Subtribe Brassicinae. Several cytoplasmic genetic markers of potential use in plant breeding programs were identified for each of the cytodemes .

1.2.3. Phylogeny and taxonomic relationships within the subtribe Brassicinae

The evidence for two distinct lineages in the subtribe indicates *polyphyletic origins* for at least two of the genera studied to date, *Brassica* and *Sinapis*. The taxonomic confusion between *Sinapis* and *Brassica* is historic (reviewed in (Baillargeon, 1986), originating with the selection of *Sinapis nigra* L. (= *Brassica nigra* (L.) W. Koch.) as the lectotype of the genus *Sinapis*. As a result, nomenclatural synonymy in these two genera, and even across other genera of the subtribe, is common. Taxonomic realignments will be required at both the generic and subtribal levels in order to more accurately reflect generic relationships. Two possible options exist:

- Expand the genus *Brassica* to include related genera, recognizing the two lineages as subgenera (note: percent divergence across the two lineages as calculated from cpDNA data is ca. 3%, which is consistent with values for other genera; or
- Redefine the genus *Sinapis* to include *S. pubescens*, *S. arvensis*, *S. alba*, and three species of *Brassica* (*B. nigra*, *B. fruticulosa*, and *B. tournefortii*). Further studies are in progress to test the monophyletic origins of other genera in the subtribe, a requirement before taxonomic revision of the subtribe can be completed.

In conclusion, a high level of congruence was observed between recognized cytodemes or crossing groups in the subtribe Brassicinae and the clusters defined by the cpDNA data. A similar congruence has been observed by (Doyle et al., 1990) for wild perennial relatives of *Glycine* subgenus *Glycine*. This correlation is significant because of the potential predictive value of cpDNA data in delimiting cytodemes and/or in detecting potentially new breeding material. The observed low levels of variation within the

cytodeme group enhance the usefulness of cpDNA data in future systematic studies of intercytodeine relationships in the subtribe.

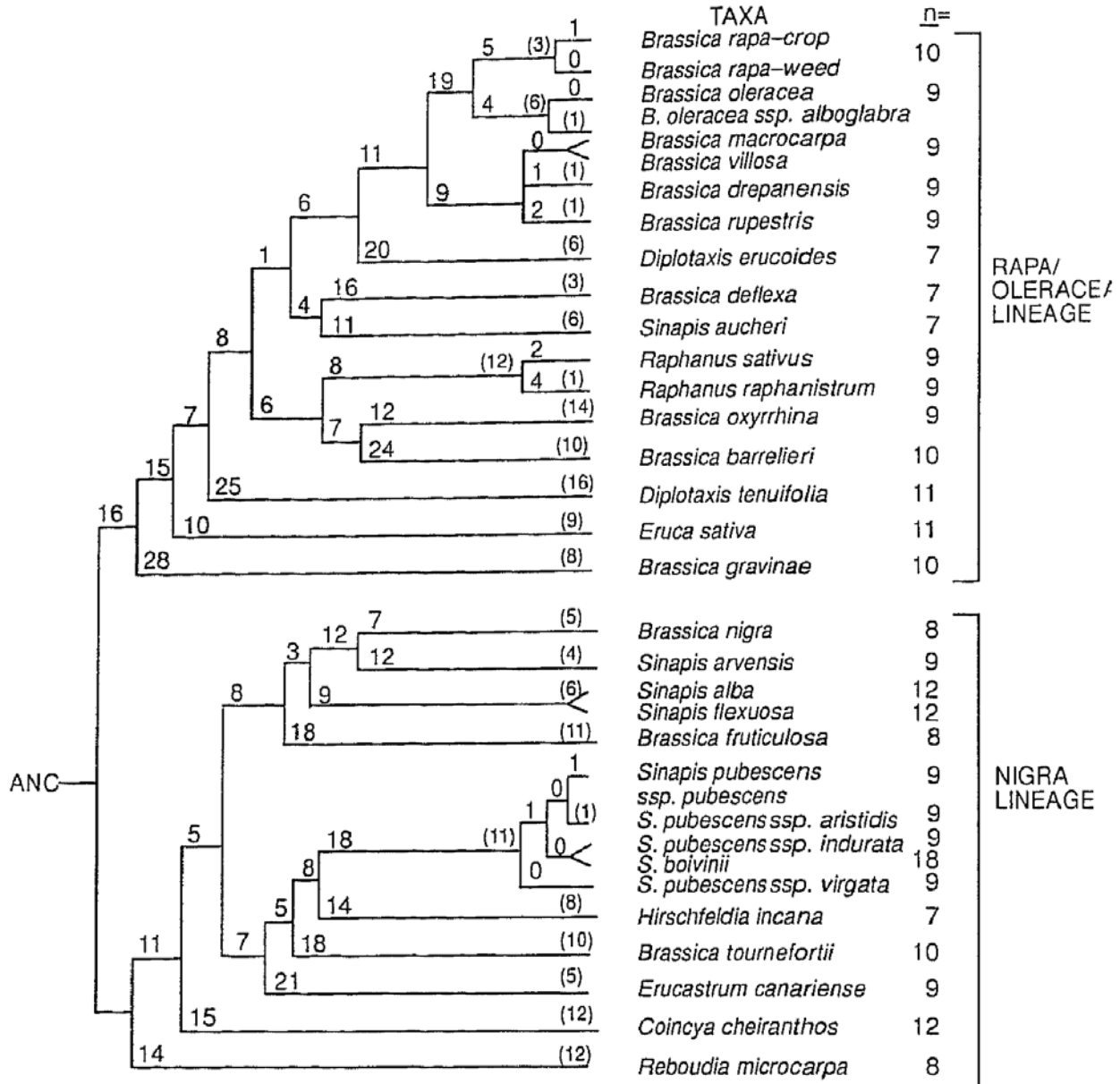


Fig. 2 Selected phylogenetic tree for the Subtribe Brassicillae based on PAUP analyses of the chloroplast DNA restriction site/length mutations in the Appendix, which are shared by two or more taxa/accessions. Tree length is 489 steps, consistency index, 0.491. Tree topology indicates how accessions are related, and branch length (numbers above the branches) indicates the minimal number of mutational steps occurring during the evolution of a particular taxon. Mutations unique to a given species and to the genus *Raphanus* (number indicated in brackets at end of branch) should be added to determine terminal branch length. ANC shows the common hypothetical common ancestor. From (Warwick & Black, 1991).

1.3 Molecular Taxonomy

Unfortunately, knowledge obtained from molecular genetics and development of *A. thaliana* is only very slowly creeping into the systematics of Brassicaceae, although one has to admit, that molecular taxonomic insights in Brassica are, compared to other genera and families, well developed, but just because of the data are abundant, they also show clearly the lacunes. Future directions of research should move beyond assessing generic relationships or limits, and should also address character development and evolution, the molecular basis of various homoplastic characters, the nature of the genome, and many other new challenges that are emerging from detailed molecular studies of *A. thaliana*. (Koch et al., 2003).

(Zhao et al., 2005) and his team studied genetic relationships within *Brassica rapa* as inferred from AFLP fingerprints:

Amplified fragment length polymorphism (AFLP) markers were employed to assess the genetic diversity amongst two large collections of *Brassica rapa* accessions. Collection A consisted of 161 *B. rapa* accessions representing different morphotypes among the cultivated *B. rapa*, including traditional and modern cultivars and breeding materials from geographical locations from all over the world and two *Brassica napus* accessions. Collection B consisted of 96 accessions, representing mainly leafy vegetable types cultivated in China. On the basis of the AFLP data obtained, we constructed phenetic trees using MEGA 2.1 software. The level of polymorphism was very high, and it was evident that the amount of genetic variation present within the groups was often comparable to the variation between the different cultivar groups. Cluster analysis revealed groups, often with low bootstrap values, which coincided with cultivar groups. The most interesting information revealed by the phenetic trees was that different morphotypes are often more related to other morphotypes from the same region (East Asia vs. Europe) than to similar morphotypes from different regions, suggesting either an independent origin and or a long and separate domestication and breeding history in both regions.

According to (Liu et al., 2006), fair meiosis in allopolyploid species could be challenged by homeologous chromosome pairing and is usually achieved by the action of homeologous pairing suppressor genes. Oilseed rape (*Brassica napus*) haploids (AC, $n = 19$) represent an attractive model for studying the mechanisms used by allopolyploids to ensure the diploid-like meiotic pairing pattern. In oilseed rape haploids, homeologous chromosome pairing at metaphase I was found to be genetically based and controlled by a major gene, PrBn, segregating in a background of polygenic variation. In this study, they have mapped PrBn within a 10-cM interval on the C genome linkage group DY15 and shown that PrBn displays incomplete penetrance or variable expressivity. The authors have identified three to six minor QTL/BTL that have slight additive effects on the amount of pairing at metaphase I but do not interact with PrBn. They have also detected a number of other loci that interact epistatically, notably with PrBn. Their results support the idea that, as in other polyploid species, metaphase I homeologous pairing in oilseed rape haploids is controlled by an integrated system of several genes, which function in a complex manner.

The family Brassicaceae is well known for its large variation in chromosome numbers, common occurrence of polyploids and many reports of interspecific gene flow. The review of (Marhold & Lihova, 2006) summarizes studies from the past decades on polyploidization and hybridization events, recognizing them as important evolutionary forces in the family. Attention is drawn to the issue of the reconstruction of reticulated pattern of evolution resulting from allopolyploid and homoploid hybrid speciation. The research of various authors on several Brassicaceae genera is presented and discussed in the context of our current understanding of polyploid and hybrid evolution. Model species, *Arabidopsis thaliana* and *Brassica* taxa, are referred to only marginally, major focus is on a comprehensive survey of studies on about a dozen best explored nonmodel genera (e.g. *Cardamine*, *Draba*, *Rorippa*, *Thlaspi*). The increasing amount of genetic and genomic resources available for Brassicaceae model species provides excellent opportunities for comparative genetic and genomic studies. Future research directions and challenges are thus outlined, in order to obtain more detailed insights into the evolution of polyploid and hybrid genomes.

According to (Lysak & Lexer, 2006) the vast genetic diversity, specific genome organization and sequencing of the *Arabidopsis thaliana* genome made crucifers an ideal group for comparative genomic studies. *Arabidopsis* genomic resources have greatly expedited comparative genomics within Brassicaceae and fostered the establishment of new *Arabidopsis* relative model systems (ARMS). The extent of genome colinearity, modes and evolutionary rates of genome alterations are being analyzed by genetic mapping with ever increasing levels of precision. Comparative cytogenetic studies in Brassicaceae are employing various chromosome landmarks and cytogenetic techniques, including localization of rDNA, variation in centromeric satellite repeats, genomic in situ hybridization (GISH), fluorescence ISH using bacterial artificial chromosomes (BAC FISH), and large-scale comparative chromosome painting. Some genome alterations may represent rare genomic changes (RGCs) and thus have the potential to resolve complex/conflicting phylogenetic relationships inferred from DNA sequencing. Comparative genomics should increasingly be integrated with molecular phylogenetics and population genetics to elucidate the processes responsible for genetic variation in Brassicaceae.

The research group of (Leflon et al., 2006) summarize their work as follows: Interspecific crosses contribute significantly to plant evolution enabling gene exchanges between species. The efficiency of interspecific crosses depends on the similarity between the implicated genomes as high levels of genome similarity are required to ensure appropriate chromosome pairing and genetic recombination. *Brassica napus* (AACC) is an allopolyploid, resulting from natural hybridization between *Brassica rapa* (AA) and *Brassica oleracea* (CC), both being diploid species derived from a common ancestor. To study the relationships between genomes of these *Brassica* species, we have determined simultaneously the pairing and recombination pattern of A and C chromosomes during meiosis of AAC triploid hybrids, which result from the interspecific cross between natural *B. napus* and *B. rapa*. Different AAC triploid hybrids and their progenies have been analysed using cytogenetic, BAC-FISH, and molecular techniques. In 71% of the pollen mother cells, homologous A chromosomes paired regularly, and usually one chromosome of each pair was transmitted to the progeny. C chromosomes

remained mainly univalent, but were involved in homoeologous pairing in 21.5% of the cells, and 13% of the transmitted C chromosomes were either recombined or broken. The rate of transmission of C chromosomes depended on the identity of the particular chromosome and on the way the hybrid was crossed, as the male or as the female parent, to *B. napus* or to *B. rapa*. Gene transfers in triploid hybrids are favoured between A genomes of *B. rapa* and *B. napus*, but also occur between A and C genomes though at lower rates.

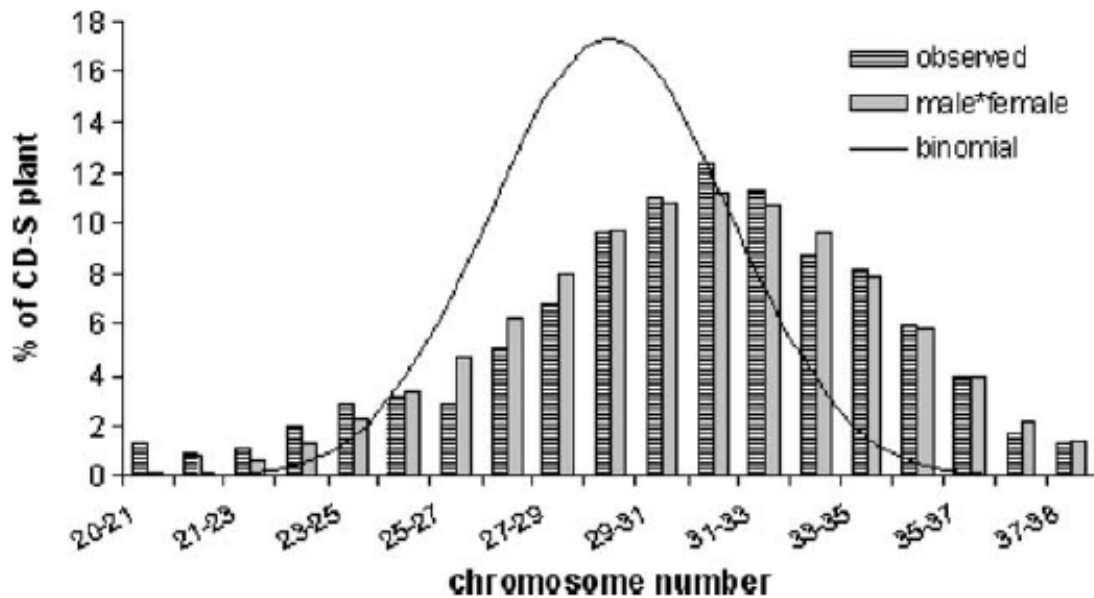
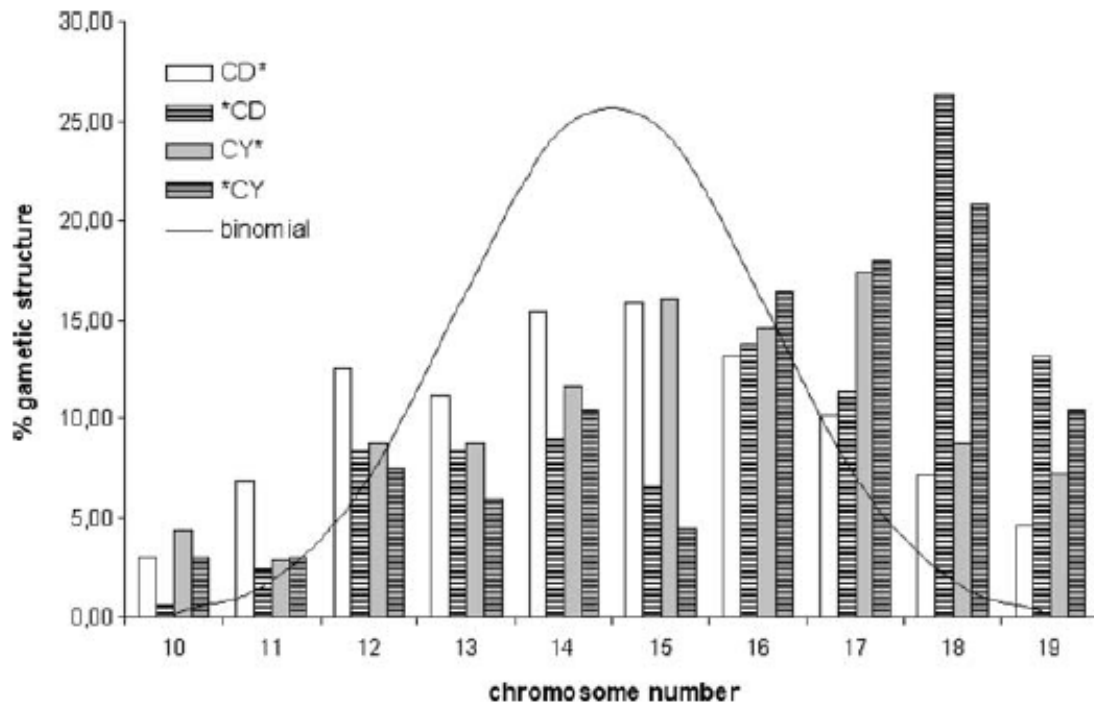


Fig. 3 above: Frequency of gametes with each chromosome number where triploid hybrids are male or female. Unreduced gametes were excluded from this analysis. Below: Distribution of chromosome number in CD-S plants

Chi-square analyses indicated that none of the observed distributions of gametic structure corresponded to expectations ($\alpha = 5\%$). The number of chromosomes transmitted to the progeny was different when the triploid hybrid was used as female versus as male (Fig. 2). When the triploid hybrid was used as female, gametes with extreme chromosome number were more represented than expected. When the triploid hybrid was used as male, gametes with 17, 18, or 19 chromosomes were highly over-represented (50.9 or 49.3% of gametes had more than 17 chromosomes in D · CD and Y · CY crosses, respectively). Neither the origin of the hybrid (CY or CD) nor that of the recurrent parent (*B. napus*, Darmor or Yudal, or *B. rapa*) had a significant effect ($\alpha = 5\%$) on the chromosome number of gametes when the hybrid was crossed as female. The average number of chromosomes transmitted by the triploid was 14.2 in the CD · C cross, 14.9 in the CD · D cross, 14.6 in the CD · Y cross, 16.1 in the D · CD cross, 15.2 in the CY · Y cross, and 15.9 in the Y · CY cross. The distribution of chromosome numbers in the CD-S progeny, obtained after selfing the CD hybrids, was different from the theoretical distribution expected if both male and female gametes fitted a binomial distribution of chromosome numbers ($P = 0.5$). The observed distribution of chromosome numbers fitted the product of the observed distribution of chromosome number in male and female gametes, which were deduced from the BC progenies (Fig. 2)

Furthermore, there would be a lot of literature to be incorporated in detailed comments: (Alemayehu & Becker, 2002; Alshehbaz, 1985; Cartea et al., 2005; Demeke et al., 1992; Dias, 1995; Diers & Osborn, 1994; Lanner, 1998; Lanner et al., 1997; Lazaro & Aguinalgalde, 1998; Marques et al., 2001; Rabbani et al., 2001; Song & Osborn, 1992; Song et al., 1988; Vonbothmer et al., 1995; Warwick & Al-Shehbaz, 2006; Warwick & Black, 1997; Warwick et al., 2006; Zeng et al., 2004; Zhao et al., 2005; Zhidkova, 1997).

2. Biosafety considerations

2.1. General remarks and gene flow

Ever since (Jorgensen & Andersen, 1994) we know about outcrossing possibilities of transgenes to wild relatives of *Brassica napus*. There is a rich bibliography on this topic.

(Ford et al., 2006) summarized the present day situation: Research on the environmental risks of gene flow from genetically modified (GM) crops to wild relatives has traditionally emphasized recipients yielding most hybrids. For GM rapeseed (*Brassica napus*), interest has centred on the 'frequently hybridizing' *Brassica rapa* over relatives such as *Brassica oleracea*, where spontaneous hybrids are unreported in the wild. In two sites, where rapeseed and wild *B. oleracea* grow together, the authors used flow cytometry and crop-specific microsatellite markers to identify one triploid F-1 hybrid, together with nine diploid and two near triploid introgressants. Given the newly

discovered capacity for spontaneous introgression into *B. oleracea*, they then surveyed associated flora and fauna to evaluate the capacity of both recipients to harm cohabitant species with acknowledged conservational importance. Only *B. oleracea* occupies rich communities containing species afforded legislative protection; these include one rare micromoth species that feeds on *B. oleracea* and warrants further assessment. It was concluded that increased attention should now focus on *B. oleracea* and similar species that yield few crop-hybrids, but possess scope to affect rare or endangered associates. Studies on gene flow within the genus *Brassica* exist in abundance:

<http://www.botanischergarten.ch/EPOBIO-Brassica/Bibliography-Brassica-Geneflow-20060430.pdf>

2.2. Competition and fitness effects

The overall present day situation in outcrossing with *Brassica napus* is summarized by (Damgaard & Kjellsson, 2005):

The introduction of genetically modified (GM) crops in the EU has raised questions concerning gene dispersal and coexistence with non-GM-farming. Quantitative estimates of the gene dispersal from fields with GM-crops to fields with conspecific non-GM-crops (conventional or organic) are therefore needed in order to suggest isolation distances and other management strategies to keep GM-pollination below acceptable threshold values. A meta-analysis of available gene-flow data for oilseed rape (*Brassica napus*) was performed. The probability distribution that seeds of non-GM-oilseed rape are fertilised by foreign pollen grains from a neighbouring field of GM-oilseed rape is modelled as functions of the width of the recipient (i.e. pollen receiving) field and the distance to the pollen donor fields. Furthermore, the significance of using a buffer zone (removal of a 1–5 m border of a recipient field parallel to the pollen donor field) to reduce GM-pollination of the crop, is quantified and discussed. The predicted median and 95% credibility level of the probability of foreign pollination is calculated as a function of the width of the recipient field and the buffer zone, as well as the distance between fields. Analysis of different management strategies shows that an increasing isolation distance is more effective to reduce GM-pollen dispersal than the use of a buffer zone, especially for small recipient fields. The analysis shows that increasing the width of a recipient oilseed rape field, relative to the pollen donor field, will have a large effect on reducing the average level of fertilisation by foreign pollen within the recipient field. The results indicate that a GM-pollination percentage <0.1% will be possible if the isolation distance exceeds 100 m and the width of the non-GM-field is larger than 200 m. If a threshold value of 0.3% is acceptable, an isolation distance of 50 m should be sufficient even for smaller fields. The use of a 5 m discarded buffer zone surrounding the non-GM-field is expected to reduce GM-pollination by about a third. The implications of the results for field management in conventional and organic farming are discussed. These figures are calculated strictly for intermixture with transgenes which have been proven harmless for consumption by regulatory authorities.

According to (Johannessen et al., 2006) interspecific F1-hybrids may arise in fields with transplastomic oilseed rape where *B. rapa* occurs as a weed. Spilled seeds, including transplastomic F1-hybrids with *B. rapa*, may germinate, which creates an opportunity for production of transplastomic BC1 with *B. rapa* as father (BC1r). Field trials were made

with three different proportions of *B. napus*, *B. rapa* and F1-hybrids and three different densities. Contrary to most studies on how plant competition affects introgression between oilseed rape and *B. rapa*, this study focused on offspring produced on F1-hybrids, where the F1-hybrids had oilseed rape as maternal parent. We estimated the BC1r production in all combinations of proportion and density, and found that *B. rapa* sired from 0.6–7.8% of the offspring. At the proportion with the highest abundance of F1-hybrids the entire paternity was assessed. There was a significant density effect on the production of BC1r but the effect differed among proportions. Both the highest and lowest frequencies of BC1r were obtained at high plant density. Neither the proportion nor density affected the number of BC1r per square-meter significantly. Biomass components decreased significantly from low to intermediate density, whereas a further increase in density only affected the thousand-seed weight significantly. On the basis of the results from the present experiment we conclude that introgression of transgenes from transplastomic oilseed rape to *B. rapa* seems most likely at current field densities of *B. napus*, and when *B. rapa* is an abundant weed.

Fitness of hybrids between genetically modified (GM) crops and wild relatives influences according to (Allainguillaume et al., 2006) potential ecological harm. They measured fitness components in spontaneous (non-GM) rapeseed × *Brassica rapa* hybrids in natural populations. The F1 hybrids yielded 46.9% seed output of *B. rapa*, were 16.9% as effective as males on *B. rapa* and exhibited increased self-pollination. Assuming 100% GM rapeseed cultivation, the authors conservatively predict < 7000 second-generation transgenic hybrids annually in the United Kingdom (i.e. ~20% of F1 hybrids). Conversely, whilst reduced hybrid fitness improves feasibility of bio-containment, stage projection matrices suggests broad scope for some transgenes to offset this effect by enhancing fitness.

Two new gene flow modelling papers show similar results with assumed and calculated safety distances well known from other publications: (Chevre et al., 2007; Devaux et al., 2007), but they also reveal the full complexity of gene flow dynamics depending on several genomic and ecological factors.

The aim of the model presented by (Chevre et al., 2007) is to describe the relationship between chromosome numbers in the progeny of a plant and the main explicative factors. For this, we have included not only the average behaviour but also the complex variability sources and the resulting variance heterogeneity. Such modelling required trial and error. For example, various transformations on the data and various regression models were considered. The resulting model allows an accurate description of the data and of its variability, and does so without relying on strong mechanistic assumptions. The general emphasis here was on finding a parsimonious empirical model rather than a complex one, while coping with the main sources of variability in the data. This empirical modelling approach is a major help for interpreting such complex data.

Our model allows statistical inference to be made and LR tests to be performed for biologically relevant hypotheses on factorial effects. Significant differences were detected for the rate of chromosome number decrease between the generations. Two main factors may explain these differences. Firstly, as some hybrids may have originated from selfing or intercrosses between partially fertile hybrid mothers, it is possible that they carry the two homologues of several oilseed rape and/or of *R. raphanistrum* chromosomes, especially when they have a high chromosome number.

When this configuration is true, pairing allows a fair transmission of the paired chromosomes to the gametes, explaining that the plants with a higher chromosome number show a slower decrease of the chromosome number in the progeny. If intercrosses occur between hybrids, the probability of occurrence of such plants with homologous chromosome pairs is higher in the first generations close to the hybrids presenting the whole genome of both species. Such homologous pairs are separated progressively in advanced generations through the recurrent pollinations with *R. raphanistrum*. This is in agreement with the individual effect of the female parent but it is important to mention that the plants with the highest chromosome number showed generally a poor fertility. Secondly, it has been established that the transmission rate of additional chromosomes may be different between chromosomes of the same genome and that the male and female transmissions can differ for the same chromosome (Chevre et al., 1997). Additionally, it is likely the transmission rate of a specific chromosome is different according to the presence or not of several other additional chromosomes. The selection pressure by the herbicide treatment did not show an effect on the decrease on the chromosome number. However, one supplementary chromosome was detected in the treated plants. Whatever the genomic structure of the plants, only those carrying the oilseed rape chromosome with the transgene will survive after herbicide treatment and that this chromosome will be retained. This difference between treated and untreated plants will disappear only after chromosome recombination between the oilseed rape chromosome carrying the transgene and a *R. raphanistrum* one.

(Devaux et al., 2007) aimed at understanding patterns of pollen movement at the landscape scale is important for establishing management rules following the release of genetically modified (GM) crops. They used a mating model adapted to cultivated species to estimate dispersal kernels from the genotypes of the progenies of male-sterile plants positioned at different sampling sites within a 10 × 10-km oilseed rape production area. Half of the pollen clouds sampled by the male-sterile plants originated from uncharacterized pollen sources that could consist of both large volunteer and feral populations, and fields within and outside the study area.

The geometric dispersal kernel was the most appropriate to predict pollen movement in the study area. It predicted a much larger proportion of long-distance pollination than previously fitted dispersal kernels. This best-fitting mating model underestimated the level of differentiation among pollen clouds but could predict its spatial structure. The estimation method was validated on simulated genotypic data, and proved to provide good estimates of both the shape of the dispersal kernel and the rate and composition of pollen issued from uncharacterized pollen sources. The best dispersal kernel fitted here, the geometric kernel, should now be integrated into models that aim at predicting gene flow at the landscape level, in particular between GM and non-GM crops.

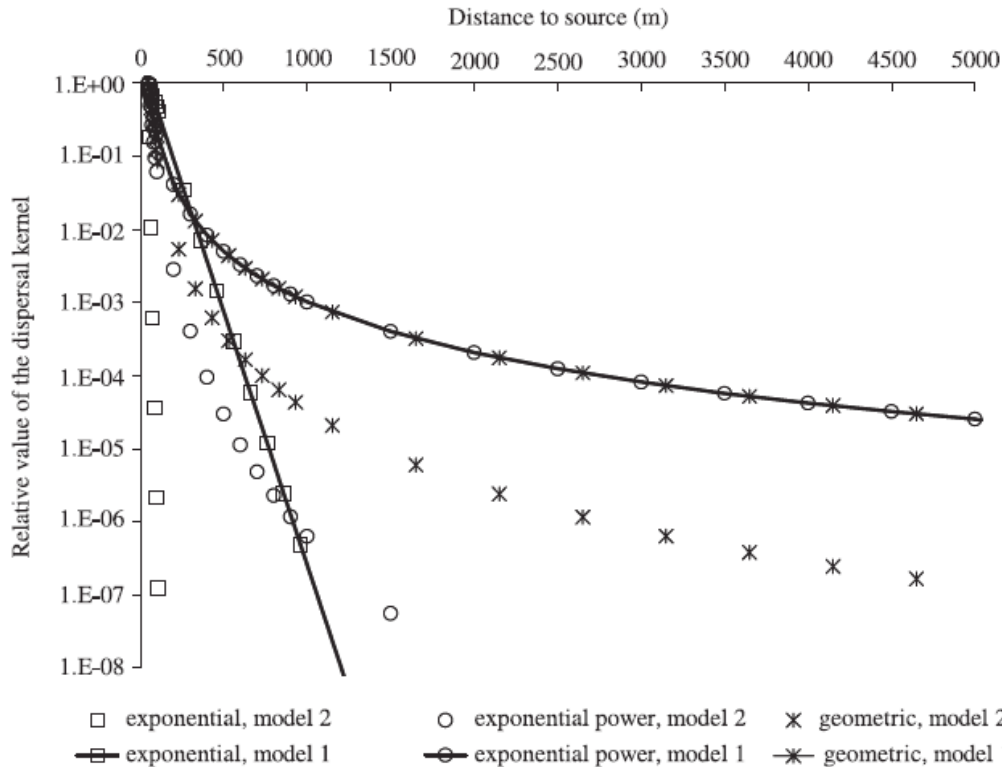


Fig. 4 Relative values of the dispersal kernels, against distance to source, estimated for models 1 and 2 on the experimental data. Open squares denote the exponential functions, open circles the exponential power functions, and crosses the geometric functions. All functions were normalized to take the same value at 50 m (see Results). From (Devaux et al., 2007)

A realistic scenario from Great Britain has been produced by (Wilkinson et al., 1993; Wilkinson et al., 2000; Wilkinson et al., 2003)

The pattern of hybridization ultimately affects the scale and rapidity of ecological change and the feasibility of containment ((Wilkinson et al., 2000). A new procedure for quantifying hybrid formation over large areas is proposed by (Wilkinson et al., 2000). They used remote sensing in order to identify possible sites of sympatry between *Brassica napus* and its progenitor species across 15 000 km² of south-east England in 1998. Two sympatric populations with *B. rapa* and one with *B. oleracea* were found over the entire survey area. Every newly recruited plant in these populations in 1999 was screened for hybrid status using flow cytometry and molecular analyses. One hybrid was observed from the 505 plants screened in the *B. rapa* populations but none of the nine *B. oleracea* recruits were hybrids. Measures to minimize gene flow are suggested, and a procedure for the post-release evaluation and containment of GM cultivars is proposed.

2.3. Mitigation of gene flow

The aim of the study of (Fargue et al., 2006) was to evaluate the interest of beginning selection process on a new genetic characteristic, cleistogamy, to manage gene flow in oilseed rape. The first step was to introduce this characteristic in an existing model of gene flow between oilseed rape populations in time and space, GENESYS-RAPE. The

second step was to evaluate the parameters of the model linked to this characteristic using field experimentations. Cleistogamous oilseed rape was shown to have an autogamy rate as high as 94% and to emit 10 times less pollen than an openflowered oilseed rape in the same conditions. But the cleistogamous character was also shown to be unstable in the genotypes tested. In a third step (Wilkinson et al., 2000) evaluated the interest of cleistogamy using simulations comparing several genotypes with or without cleistogamy in two different cropping systems. These simulations showed that an oilseed rape both dwarf and cleistogamous was interesting to limit gene escape and that a 99%-autogamous oilseed rape was interesting to limit both gene escape from and harvest contamination of the 99%-autogamous oilseed rape.

A surveillance scheme is proposed by (Pekrun et al., 2006):

Persistence of oilseed rapeseed in soil can result in weed problems but also reduce oil quality of following rape crops or result in unwanted gene escape which is particularly relevant in the context of genetically modified oilseed rape. In this paper data from 13 field experiments at sites in England, Austria and Germany are presented where tillage operations were tested that potentially reduce the build-up of a seed bank. In the majority of experiments seed losses were artificially simulated by broadcasting ca. 10,000 freshly ripened rapeseed m⁻² onto cereal stubbles. Oilseed rapeseedlings in autumn, the seed bank in winter-spring and yields of the following crop winter wheat were assessed as a function of tillage regime. During summer and autumn 19-70% of the seeds germinated and emerged. This part of the population was killed by following tillage operations or herbicide applications. However, 0-29%, in moist years 0-5%, of the initially broadcasted seeds developed dormancy and remained ungerminated in the soil until the following winter-spring. Delaying incorporation of the seeds by leaving the stubble untouched for up to 4 weeks resulted in a reduced seed bank in almost every case. Also, repeated stubble tillage compared to an early single stubble tillage operation resulted in a smaller seed bank. The type of primary tillage (ploughing versus non-inversion cultivation) had no clear effect. No relation was found between the number of seedlings in autumn and the size of the seed bank the following winter-spring. Grain yield of the following crop winter wheat was not adversely affected by delayed stubble tillage. The results indicate that stubble tillage aiming at a reduced seed bank of oilseed rape should focus on conditions avoiding induction of secondary dormancy rather than improving germination conditions. *This means that, under the climatic conditions of central and western Europe, the stubble should be left untouched for several weeks after harvest before starting the usual tillage sequence with stubble tillage and ploughing or a non-inversion tillage sequence.*

A simple mechanistic model is presented by (Pekrun et al., 2005) which describes population dynamics of volunteer oilseed rape within a field. The model calculates the number of volunteers appearing in each crop and the seedbank after each crop. The main input variables are harvesting losses when the crop is oilseed rape, crop rotation, soil cultivation, soil moisture content within the arable soil layer and the level of volunteer control in each crop in the rotation. Simulation studies suggest that there are a number of agronomic means of minimising volunteer oilseed rape populations effectively. The amount of harvesting losses, the time span between oilseed rape harvest and the first tillage operation post-harvest, the efficiency of controlling oilseed rape in other crops and rotation itself are key components of a programme for ensuring that volunteer oilseed

rape populations are minimised. Simulation runs showed that the proportion of volunteer plants within a crop of oilseed rape will be relatively high, even though the density of volunteers is low in other crops. This contamination of a rape crop could be a particular problem in the context of the cultivation of genetically modified rape. The model would benefit from improved estimates of some parameters. More data are particularly necessary on the long-term development of a seedbank of oilseed rape and the relationship between the size of the seedbank and the number-of volunteers in various crops.

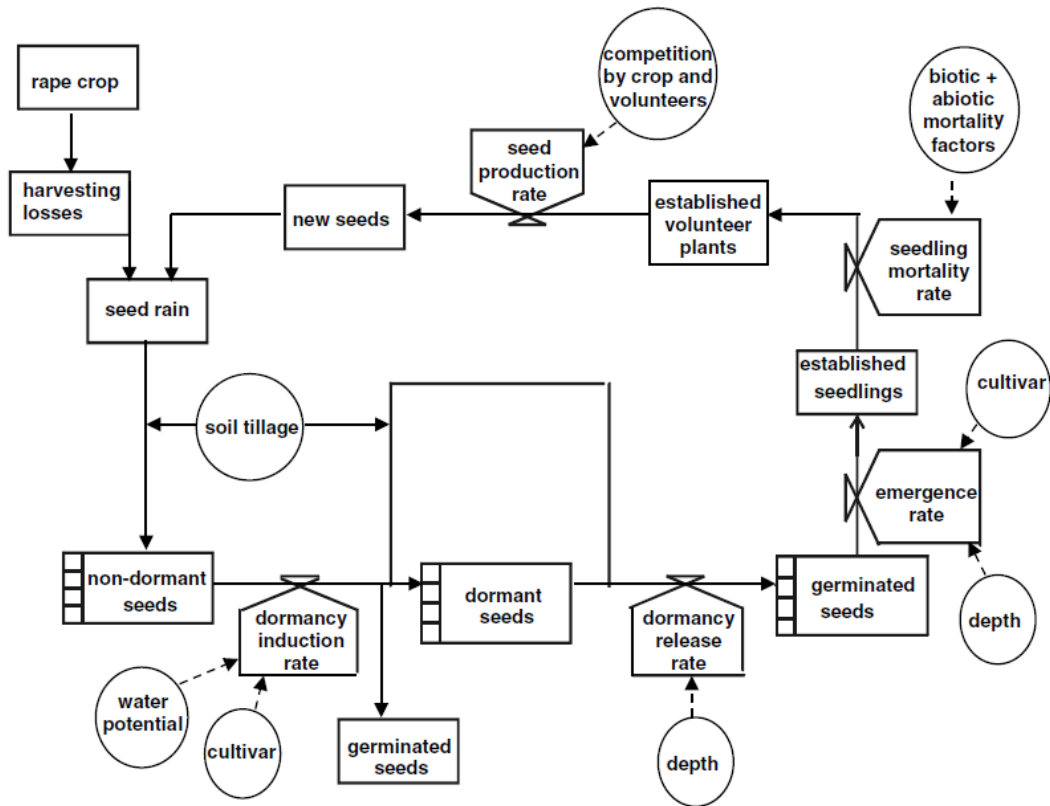


Fig. 5 General structure of the model. Boxes represent individuals (seeds or volunteer plants). Values represent rates that influence the transition from one step to the next. Within the circles factors are depicted that influence transition rates.

Strand Question	Score
CPW Propensity for successful pollen-mediated gene flow between the crop and wild relatives	
CPW1 Do interfertile wild relatives of this crop exist ? (0/1)	1
CPW2 Is there a probability that the crop will flower and produce viable pollen during its cultivation? (0/1)	1
CPW3 Upon flowering, is 95% of the crop pollen deposited within 1 m (1), 10 m (2), 50 m (3), 100 m (4), 250 m (5) or 500 m (6)? (1-6)	6 minimal
CPW4 If flowering does occur is the wild relative in question rated as an obligate inbreeder (0), a partial inbreeder/outbreeder (1) or an obligate outbreeder (2)? (0-2)	1
CPW5 If fertilization is achieved by the deposited pollen, will a viable F ₁ hybrid individual establish itself? (0/1)	1

Strand Question	Score
CPC Propensity for successful pollen-mediated gene flow between the crop and related commercial varieties	
CPC1 Probability that crop flowers and produce viable pollen during its cultivation? (0/1)	1
CPC2 Upon flowering, is 95% of the crop pollen deposited within 1 m (1), 10 m (2), 50 m (3), 100 m (4), 250 m (5) or 500 m (6)? (1-6)	6 minimal
CPC3 If flowering occurs is receptive crop rated as an obligate inbreeder (0), a partial inbreeder/outbreeder (1) or an obligate outbreeder (2)? (0-2)	1
CPC4 If fertilization happens, will a viable F ₁ individual be established itself from hybrid seed in absence of mechanical/chemical control? (0/1)	1

CSV Propensity for successful seed- and tiller mediated gene flow from commercial crop to volunteer	
CSV1 Does the crop produce seed during its cultivation? (0/1)	1

CSV2	Post-harvest, will the seed survive and germinate within the confines of a managed field? (0/1)	1
CSV3	Will the volunteer develop into a viable individual? (0/1)	1
CSF	<i>Propensity for successful seed- and tiller mediated gene flow from commercial crop to feral</i>	
CSF1	Does the crop produce seed during its cultivation? (0/1)	1
CSF2	Following transfer from cultivation site: will wayward seed survive and germinate? (0/1)	1
CSF3	Will the resulting individuals establish into a viable feral population? (0/1)	1
CGC	Consequences of Gene Flow	
CGC1	Does crop mix with related crop traits and jeopardize harvest value ? (0/1)	1
CGC2	Does crop mix with wild relatives or feral traits and pose an biodiversity threat ? (0/1)	1
CGC3	Does crop produce volunteers and/or seed deposits and pose an environmental threat ? (0/1)	1
CMG	Mitigation Gene Flow crop to crop, crop to wild relative and crop to feral	
CMG1	Mitigation with safety distances possible (J/N) 1 m (1), 10 m (2), 50 m (3), 100 m (4), 250 m (5) or 500 m (6)? (J/N) (1-6)	6 minimal (J)
CMG2	Mitigation with molecular safety measures apomixis AP, cytoplasmatic sterility CS, tandem construc TA, gene switching GS AP, TA, GS, (0/1)	AP, CS, TA, GS, 1

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