on biodiversity and geneflow of selected biofuel crops

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Avena, Beta vulgaris, Brassica napus, Crambe, Linum, Miscanthus, Nicotiana tabaccum, Populus, Salix, Triticum

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Avena, Oats

1. Taxonomy

(Grebenstein et al., 1998) in his general overview on the taxonomy of Aveneae wrote: In accordance with current systematic opinions, the tribe *Aveneae* is closely associated with the tribe *Poeae.* Both tribes can be regarded as monophyletic sistergroups (Fig. 2) with a common, monophyletic origin in the grass subfam. *Pooideae* as already indicated by chloroplast DNA restriction site variation (Soreng et al., 1990), by ITS (= sequencing of nuclear ribosomal DNA) (Barker et al., 2001; Hsiao et al., 1994, 1995a, b; Hsiao et al., 1998; Hsiao et al., 1999), and, though less extensively sampled, by *rbcL* sequence data (Duvall & Morton, 1996). A completely different systematic view was expressed in the last systematic review of grasses published by (Tsvelev, 1989) who argued that separation of the tribes *Poeae* and *Aveneae* is based on comparatively weak morphological characters and consequently he summarised *Poeae* and *Aveneae* under a broad tribe *Poeae.* This unconventional suggestion should be kept in mind when further molecular data of phylogenetically critical taxa become available by molecular work. The tribe *Triticeae* (represented here by *Secale cereale)* and the *Bromeae (Bromus inermis)* are rather distantly related to *Poeae* and *Aveneae* species as indicated by ITS sequence data (Hsiao et al., 1995a, b), studies based on chloroplast DNA restriction fragment length polymorphisms (Soreng, 1990),(Kellogg, 1992), and by morphological evidence (Clayton & Renvoize, 1986).

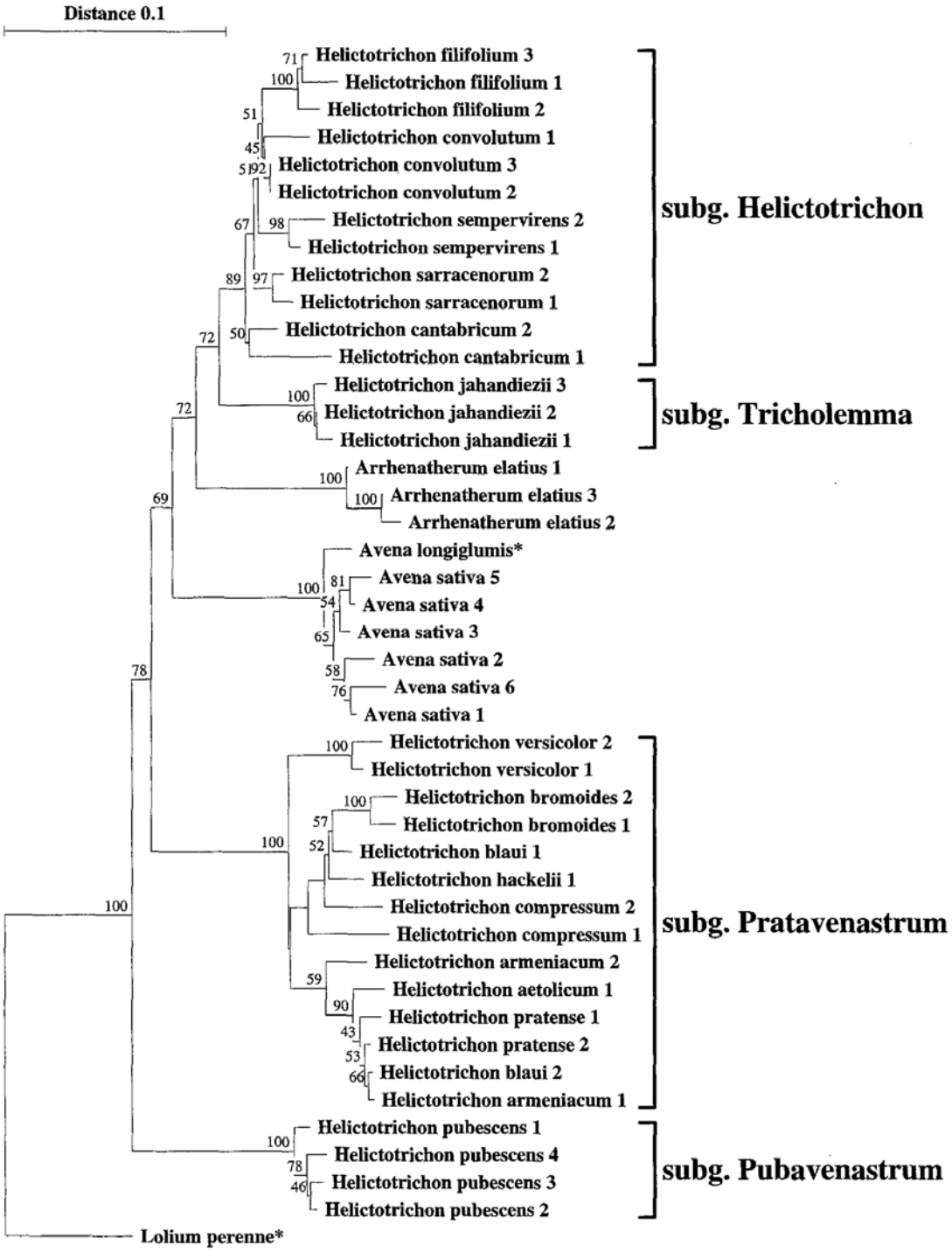


Fig. 1 Neighbor-joining tree inferred from rDNA ITS sequences in 'core genera' of *Aveneae* subtribe *Aveninae* analysed with the distance matrix method. Numbers above branches are bootstrap values. Branch lengths are proportional to distance. Outgroup: *Lolium perenne (Poeae).* Asterisk designates sequence data taken from (Hsiao et al., 1994, 1995a, b), out of (Grebenstein et al., 1998).

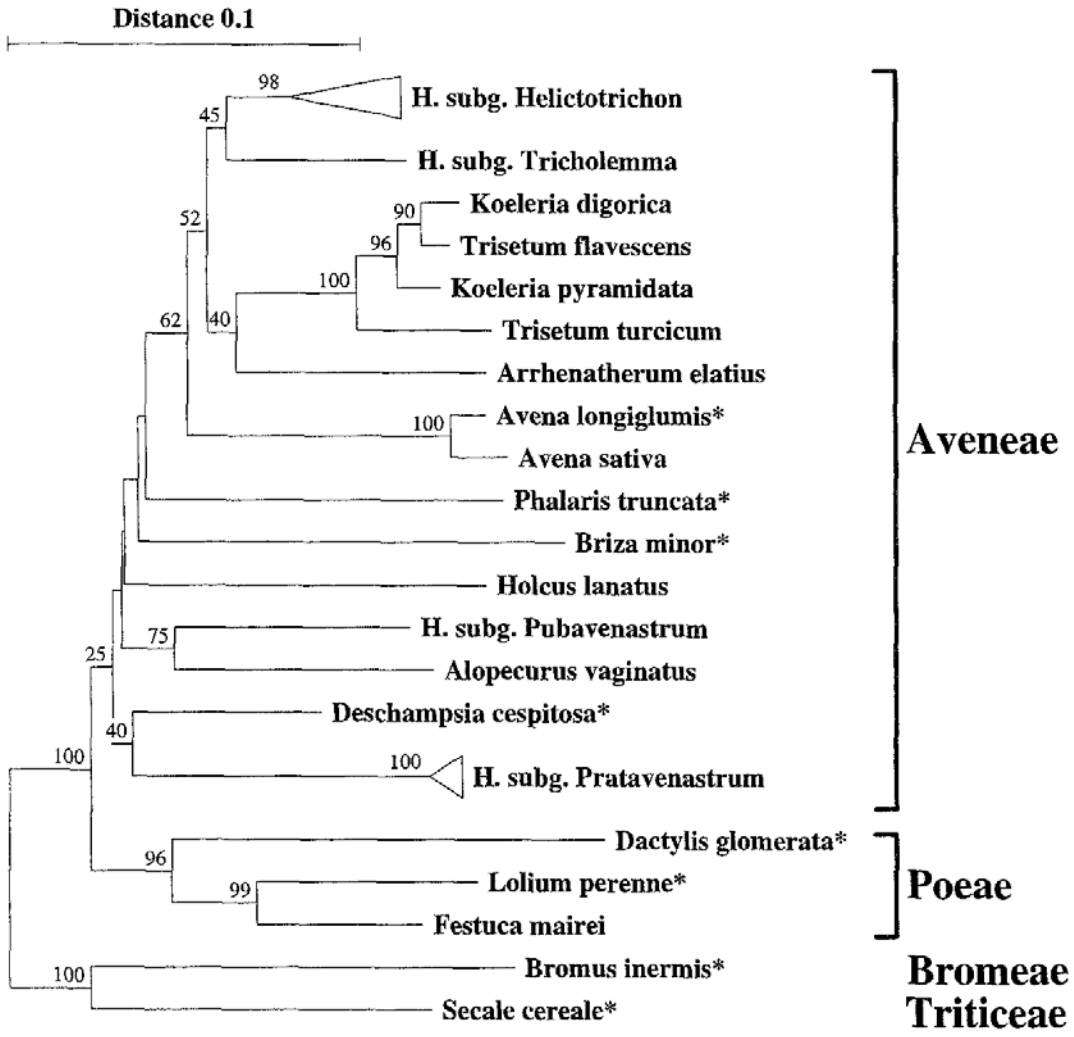


Fig. 2 Phylogenetic tree inferred from ITS sequences of 15 genera of tribes *Aveneae* and *Poeae* generated by the neighbor-joining distance matrix method. Numbers above branches are bootstrap values. Branch lengths are proportional to distance. Outgroups: *Bromeae (Bromus inermis)* and *Triticeae (Secale cereale).* Asterisk designates sequence data taken from (Hsiao et al., 1994, 1995a, b)

In conclusion, the molecular analysis of ITS sequences of several taxa of the *Aveneae* suggests that the ancestry of the agronomically important genus *Avena* has to be sought within comparatively small-flowered *Aveneae* taxa genus *Arrhenatherum* and small-flowered subgenera of *Helictotrichon* are close extant relatives of *Avena,* genus *Helictotrichon* is para- if not polyphyletic, genera *Trisetum, Koeleria* and probably others form a separate lineage characterised by a particular 9-bp deletion the delineation of some genera and subtribes of *Aveneae,* and perhaps tribes of subfam. *Pooideae* needs to be reevaluated by including phylogenetically critical taxa and combining orphological, anatomical and molecular datasets.

(Beer et al., 1993) demonstrate, that genetic patterns revealed by proximity coefficients may be affected by choice of traits, method of scoring, any subsequent transformations of scores, and choice of proximity coefficient. Their objective was to compare restriction

fragment length polymorphism (RFLP), isozyme polymorphism, and variation in qualitative and quantitative morphological traits in a geographically stratified set of 177 accessions of a hexaploid wild oat, Avena sterilis L. Jaccard similarity coefficients (SJ) and Russell and Rao similarity coefficients (SRR) were calculated for all pairs of genotypes from restriction fragments and, separately, from isozymes. Standard taxonomic (DSZ), Mahalanobis, and Good mean distances were calculated from 26 morphological trait scores. Clustering of mean DSZ values or mean SJ(RFLP) values between pairs of countries produced similar dendrograms while SRR(isozymes) values resulted in different subgroups. Rankings of within-country diversity were similar for different types of traits but were unrelated to geographic proximity of provenances of the accessions. Proximity coefficients based on the same type of trait (either RFLP, isozyme, or morphology) were highly correlated (Mantel statistic) (0.6-0.9),while correlations for proximities based on different types of traits were less than or equal to 0.35. Isozyme and RFLP-based proximities were poorly correlated, and both were poor predictors of morphological relationships with the highest correlation being - 0.35 between SJ(RFLP) and DSZ. While broad patterns of variation revealed by different types of traits were similar in this sample of A. sterilis, differences in pairwise estimates of relationship were sufficiently great to question the exclusive use of one type of trait for sampling and management of plant germplasm collections.

2. Biosafety considerations

Outcrossing of wild oats has been systematically studied in California by (Imam & Allard, 1965), there findings summarized:
Quantitative estimates were made of the extent of self-fertilization and the amount of genetic variability in natural populations of wild oats from three regions of central California. Seed parents were chosen from seven sites within these regions and the progenies of these plants were grown in replicated field plantings at Davis, California. Individual plants were scored for two monogenic characters and measured for five quantitative characters. Estimates of outcrossing based on the monogenic characters varied from 1 to 12 percent. The frequencies of genotypes for the monogenic characters indicated that high reproductive advantage is associated with heterozygosity for the chromosome segments marked by these loci.

Three sources of genetic variability could be distinguished and measured for the quantitative characters: differences between regions, differences between sites within regions, and plant-to-plant differences within sites. The measurements indicated that wild oats are differentiated geographically and the diff erentiations observed were those one would expect to permit each population to meet the requirements imposed by its habitat.

Families derived from the same site showed a wide range of means for various characters, indicating that populations of wild oats include plants of many different genotypes. There was also substantial variability within families derived from single plants taken from natural populations. Response to selection in plus 62 and minus directions established that part of this within-family variability is genetic and that many if

not all individuals in natural populations are heterozygous at numerous loci. Comparisons between the progeny of selfed and openpollinated seeds from single plants suggested that mild loss in vigor accompanies in breeding. (Imam & Allard, 1965)

The results indicated that the genetic system of wild oats allows for continued repatterning of the gene pool in a manner differing only in degree from outbreeding species and at the same time provides for perpetuation of superior genotypes along the pattern postulated for inbreeding species in which the population is comprised largely of arrays of coexisting homozygous genotypes. The genetic system of wild oats thus appears to combine much of the flexibility of outbreeders with some of the ability of inbreeders to maintain specific highly adapted genotypes. It was postulated that the flexibility of the genetic system of wild oats has contributed to the success of this species in occupying complex habitats.

Outcrossing in Avena sativa L. have been reported to range from 0 to 9.8%, but mostly it was less than 1%, (Jensen, 1966; Schemske & Lande, 1985). (Thurmann & Womak, 1961) and (Grindeland & Frohberg, 1966) obtained up to 2.4% and 6% outcrossing, and 0.5% to 2.4% in the checks respectively, following mutagen treatments of A. sativa, although (McKenzie et al., 1975) found less than 1.0% outcrossing following such treatments. Outcrossing in Avena sterilis L. - Avena fatua L. natural mixed populations, measured by the number of plants having an A. sterilis phenotype among plants derived from the A. fatua parent, was 4.8 and 1.2% in 2 consecutive years. (Shorter et al., 1978). (Caldecott et al., 1959) and (Konzak, 1959) concluded that field hybridization of oats is increased on plants grown from irradiated seeds, and (Mikaelsen & Aastveit, 1957) found the fertility of oat plants was reduced from 99 to 27% by seed irradiation. (Thurmann & Womak, 1961) reported that irradiation of oat seeds with X-rays and thermal neutrons increased the outcrossing, and (Weber & Hanson, 1961) found that irradiation of seeds increased outcrossing in soybeans from 4 to 6 times.

(Grindeland & Frohberg, 1966) estimated the degree of outcrossing in selfed progeny of the F1 and successive backcross generations in four A. sativa x A. sterilis matings. They also considered effects of various natural crossing rates on maintenance of heterozygosity.

Outcrossing percentages were estimated in 1F, oat populations from four matings of Avena satwa L. x A. sterilis L. The populations were generated from the original cross and backcross 1 to 5 to the A. *sativa* parent in each mating. Mean outcrossing averaged across generations ranged from 1.8 to 8.7% for the four matings. Outcrossing values were 2.8, 10.7, 9.1, 4.7, 2.4, and 4.9% in the Bc_0, Bc_1, Bc_2, Bc_3, Bc_4, and Bc_5 - generations respectively. Calculations showed that outcrossing in oats has a much greater relative effect on retaining heterozygosity in the F_5 to F_{10}, generations than it has in F_1 to F_{10}.

Two separate field experiments were conducted by (Murray et al., 2002) to quantify the degree of plant-to-plant outcrossing and pollen-mediated gene flow (PMGF) in wild oat. The purpose of the study was to determine the extent to which pollen movement could contribute to the spread of herbicide resistance in this species. In both experiments, an acetyl-CoA carboxylase inhibitor-resistant (R) wild oat genotype (UM1) was used as the

pollen donor and a susceptible (S) genotype (UM5) was used as the pollen receptor. Hybrid progeny resulting from a cross between UM1 and UM5 were identified using the herbicide resistance trait as a marker. In the plant-to-plant outcrossing experiment, single UM5 plants were closely surrounded by 20 homozygous R UM1 plants in hills. By screening seed from the S parent for resistance, outcrossing was determined to range from 0 to 12.3%, with a mean of 5.2% over 10 hills. In the PMGF experiment, single homozygous R UM1 plants were surrounded by UM5 plants arranged in a hexagonal pattern at low and high densities (total of 19 and 37 wild oat plants m(-2)), growing within spring wheat and flax crops. In the wheat crop, mean wild oat outcrossing was 0.08 and 0.05% at low and high densities, respectively. In the less competitive flax, corresponding outcrossing values were 0.07 and 0.16% at low and high densities, respectively. Distance from the pollen source was a significant factor only for the high-density planting arrangement in flax. Up to 77 R hybrid seeds were recovered from 6 m^2 in the PMGF experiment, indicating that PMGF contributes to the evolution of resistance in wild oat populations. However, the contribution of pollen movement to resistance evolution and the spread of resistance in wild oat populations would be relatively small when compared with R seed production and dispersal from a resistant plant.

Finally, a study on modelling outcrossing behaviours of Avena fatua and barbata have been assessed by (Damgaard, 2002), which describe the most likely long-term ecological scenario that Avena fatua would out-compete Avena barbata. Interestingly enough they found that the calculated posterior probabilities are relatively independent of the probability of germination, establishment, and reaching reproductive age as long as they have the same relative magnitude.

3. Transgenic oats

Transgenic Avena have already been contructed:

According to (Pawlowski & Somers, 1998; Pawlowski et al., 1998) Transgenic DNA integrated into the oat genome is frequently interspersed by host DNA.

(Zhang et al., 1999) transformed commercial oats ‘Garry’ and barley cv ‘Harrington’ using shoot meristematic cultures (SMCs) derived from germinated seedlings. Six-month-old SMCs of oat were induced on MPM and bombarded with *bar* and *uidA*; 9-month-old SMCs of barley were induced on an improved medium (MPM-MC) containing maltose and high levels of copper and bombarded with *bar/nptII* and *uidA*. After 3–4 months on selection, seven independent transgenic lines of oat were obtained, two lines of barley. All transgenic lines produced T_0 plants; five lines of oat and one line of barley were self-fertile. Both Mendelian and non-Mendelian segregation ratios of transgene expression were observed in T_1 and T_2 progeny of transgenic oat. Normal as well as low physical transmission of the transgenes was also seen in T_1 and T_2 progeny of oat. The *bar*-containing line of barley showed stable transgene expression in all of the T_1 and T_2 progeny tested.

(Leggett et al., 2000) report on Avena transformed with gus and bar transgenes. Fluorescence in situ hybridization (FISH) was used to localize two transgenes (gus and bar), carried on plasmids pACT-1F and pUBA, respectively, on mitotic metaphase squashes of T1 plants of the cultivated hexaploid oat Avena sativa L. cotransformed by microprojectile bombardment of embryogenic callus. Among the eight progeny analysed by FISH in each of two lines, they detected plants null, hetero- and homozygous for the two genes in one line, and plants null and heterozygous for the two genes in the other line. Their results demonstrated that in the two independent transformation events, the gus and bar genes had inserted in the same position relative to each other. In each transformation event, the insertions occurred on D satellite (SAT) chromosomes bearing a C genome translocation. See fig. 3 below.

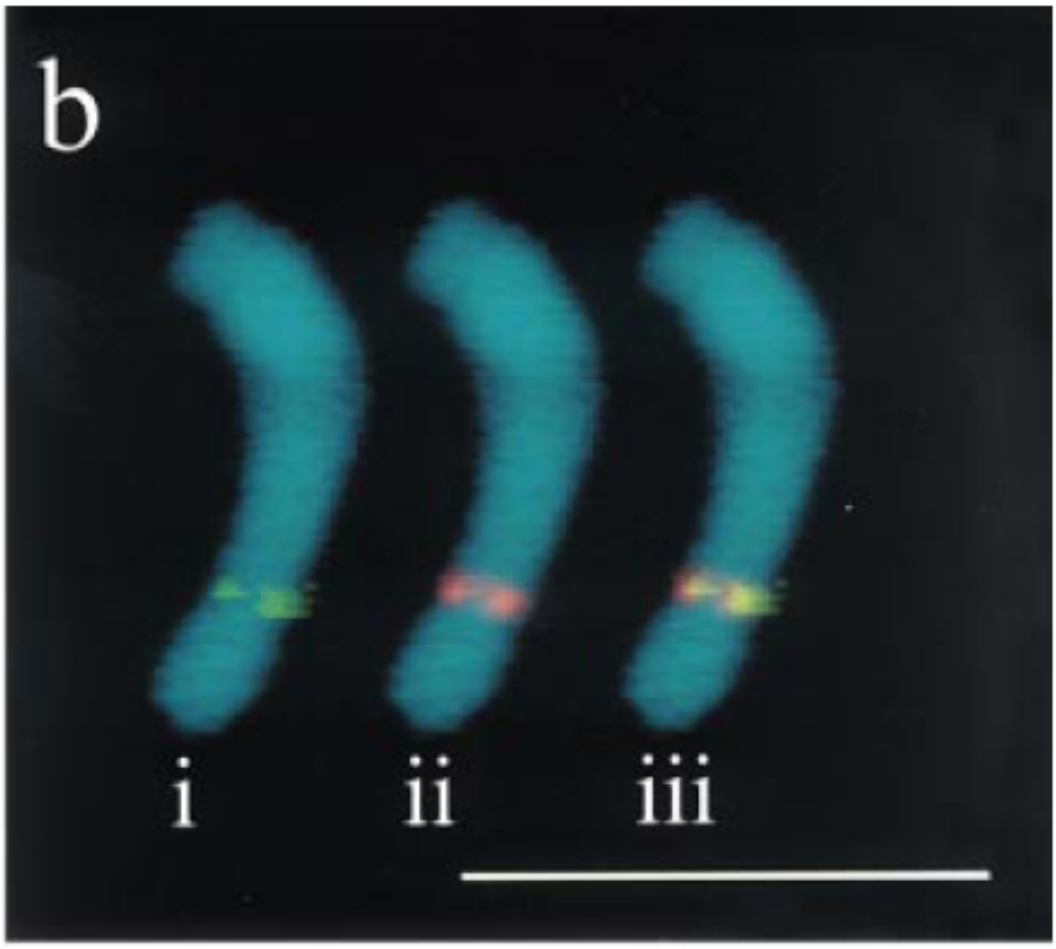


Fig. 3 Mitotic metaphase squash of line D1.3, probed with labelled pUBA (green) and pACT1-F (red) showing that both transgenes have integrated at the same site (arrowhead). (b) The transformed chromosome from (a) enlarged, showing the independent signal from: (i) the pUBA probe (green); (ii) the pACT1-F probe (red); and (iii) the combined signals from both probes.

According to (Svitashev et al., 2000; Svitashev et al., 2002), the structure of transgene loci in six transgenic allohexaploid oat (*Avena sativa* L.) lines produced using microprojectile bombardment can be characterized using fluorescence in situ hybridization (FISH) on extended DNA fibers (fiber-FISH). The transgene loci in five lines were composed of multiple copies of delivered DNA interspersed with genomic DNA fragments ranging in size from ca. 3 kb to at least several hundred kilobases, and in greater numbers than detected using Southern blot analysis. Although Southern analysis predicted that the transgene locus in one line consisted of long tandem repeats of the delivered DNA, fiber-FISH revealed that the locus actually contained multiple genomic interspersions. These observations indicated that transgene locus size and structure were determined by the number of transgene copies and, possibly to a greater extent, the number and the length of interspersing genomic DNA sequences within the locus. Large genomic interspersions detected in several lines were most likely the *products of chromosomal breakage induced either by tissue culture conditions or, more likely, by DNA delivery into the nucleus using microprojectile bombardment*. The authors propose that copies of transgene along with other extrachromosomal DNA fragments are used as patches to repair double-strand breaks (DSBs) in the plant genome resulting in the formation of transgene loci.

(Perret et al., 2003) transformed by particle bombardment of primary embryogenic callus Two oat varieties, Melys (spring variety) and Bulwark (winter variety) using either a ubi-bar-ubi-gus co-integration vector or co-transformed (Melys) with a ubi-bar plasmid together with one of three plasmids containing the -glucuronidase (gus) gene under the control of either a rice actin promoter, a CaMV35S promoter or a wheat high molecular weight glutenin promoter. Morphologically normal and fertile transgenic plants were regenerated following callus selection with glufosinate ammonium. Evidence for the integration and functioning of the selectable (bar) and reporter (gus) genes in To and T1 plants was confirmed by PCR, Southern hybridisation, fluorescence in situ hybridisation (FISH), histochemical assays, and by progeny analysis. Transformation rates varied from 0.2 to 5.0 lines/plate of callus bombarded, with co-transformation frequencies of 83 to 100 %, and co-expression frequencies of 60 to 100 %. Copy numbers for the bar and gus gene varied from 3 to 17 and from 2 to 20 respectively. Cell and tissue specific expression of the gus gene was evident from the different promoters, with the HMW glutenin promoter showing endosperm specific expression in T1 seed. No expression of the gus gene under the CaMV35S promoter was detected in any tissues. Progeny analysis provided evidence of Mendelian inheritance of the introduced genes suggesting either one or two unlinked integration sites. This was confirmed by fluorescence in situ hybridisation to chromosome spread preparations. No segregation of the gus gene from the bar gene was observed in any of the progeny derived from co-transformation.

(Pawlowski et al., 1998) published about an irregular pattern of transgene silencing which they revealed in expression and inheritance studies conducted over multiple generations following transgene introduction by microprojectile bombardment of allohexaploid cultivated oat (Avena sativa L.). Expression of two transgenes, bar and uidA, delivered on the same plasmid was investigated in 23 transgenic oat lines. Twenty-one transgenic lines, each derived from an independently selected transformed tissue culture, showed expression of both bar and uidA while two lines expressed only

bar. The relationship of the transgenic phenotypes to the presence of the transgenes in the study was determined using
phenotypic scoring combined with Southern blot analyses of progeny,
coexpression of the two transgenic phenotypes since the two transgenes always cosegregated, and
reactivation of a transgenic phenotype in self-pollinated progenies of transgenic plants that did not exhibit a transgenic phenotype.
Transgene silencing was observed in 19 of the 23 transgenic lines and resulted in distorted segregation of transgenic phenotypes in 10 lines. Silencing and inheritance distortions were irregular and unpredictable. They were often reversible in a subsequent generation of self-pollinated progeny and abnormally segregating progenies were as Likely to trace back to parents that exhibited normal segregation in a previous generation as to parents showing segregation distortions. Possible causes of the irregular patterns of transgene silencing are discussed.

(Makarevitch et al., 2003) write: A substantial literature exists characterizing transgene locus structure from plants transformed via Agrobacterium and direct DNA delivery. However, there is little comprehensive sequence analysis of transgene loci available, especially from plants transformed by direct delivery methods. The goal of this study was to completely sequence transgene loci from two oat lines transformed via microprojectile bombardment that were shown to have simple transgene loci by Southern analysis. In line 3830, transformed with a single plasmid, one major and one of two minor loci were completely sequenced. Both loci exhibited rearranged delivered DNA and flanking genomic sequences. The minor locus contained only 296 bp of two non-contiguous fragments of the delivered DNA flanked by genomic (filler) DNA that did not originate from the integration target site. Predicted recognition sites for topoisomerase II and a MAR region were observed in the transgene integration target site for this non-functional minor locus. Line 11929, co-transformed with two different plasmids, had a single relatively simple transgene locus composed of truncated and rearranged sequences from both delivered DNAs. The transgene loci in both lines exhibited multiple transgene and genomic DNA rearrangements and regions of scrambling characteristic of complex transgene loci. The similar characteristics of recombined fragments and junctions in both transgenic oat lines implicate similar mechanisms of transgene integration and rearrangement regardless of the number of co-transformed plasmids and the level of transgene locus complexity.

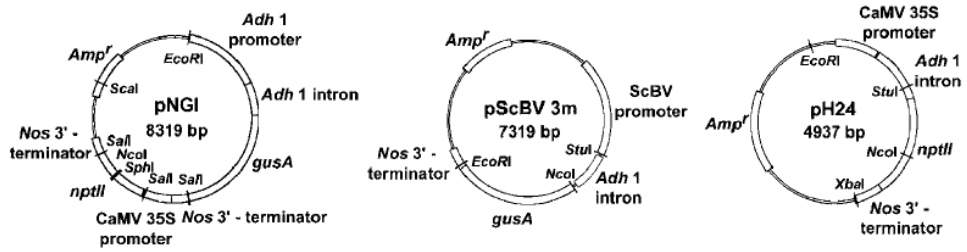


Fig. 4 Maps of plasmids used to produce transgenic oat lines 3830 (pNGI) or 11929 (pH24 and pScBV 3m). Restriction sites used in the analysis are shown. From (Makarevitch et al., 2003)

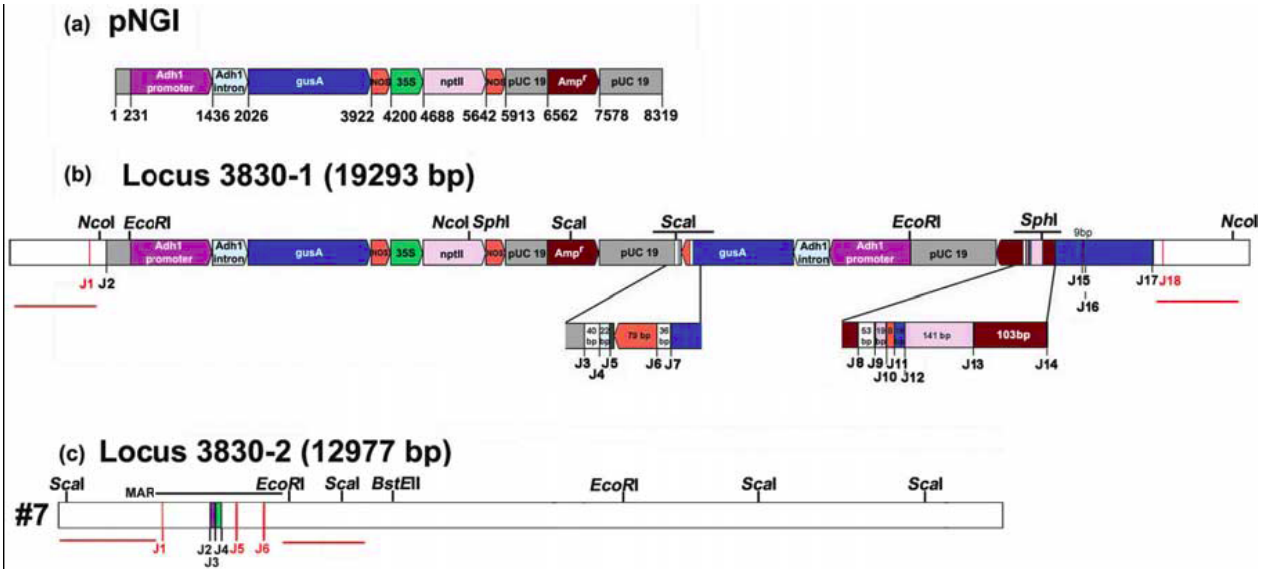


Fig. 5 Summary of sequence analysis of transgene locus clones isolated from transgenic line 3830. a. Linear map and structural components of pNGI used in producing transgenic line 3830. Colors indicate the structural components of pNGI. b. Structures of locus 3830-1 and ten lambda clones isolated from line 3830 corresponding to the main transgene locus 3830-1. Colors indicate transgene sequences that are identical to structural components of pNGI. White boxes indicate unknown sequences that are presumably oat genomic DNA. Black vertical arrows denoted by 'J' indicate junctions between noncontiguous transgene fragments or between transgene and genomic DNA. Red vertical arrows denoted by 'J' indicate the deduced position of junctions between noncontiguous genomic DNA fragments. Black bars above loci represent the regions of extensive scrambling that were PCR-amplified and sequenced from total genomic DNA for the structure verification. Red bars under loci represent regions of genomic DNA that were used as probes for hybridizations. c. The structure of lambda clone 7 isolated from line 3830 and corresponding to one of the minor transgene loci designated as 3830-2. A matrix attachment region is designated as MAR. Colors, bars, and arrows are coded as in b. From (Makarevitch et al., 2003)

4. Management and mitigation of gene flow

(Cavan et al., 2001) studied the case of Avena fatua coming up in wheat cultures, and proposed management methods in order to avoid herbicide tolerant wild oat. This paper demonstrates the management problems involved in cultivating transgenic oats, since wild oat is present all over Europe and needs to be taken into account. Consequently, from these management proposals one can learn for cultivation transgenic non-food oats.

A striking feature of the model the authors used is that good weed control is achieved for a number of years, but once this is lost, the resistant population increases very rapidly (Figures 1 and 2).

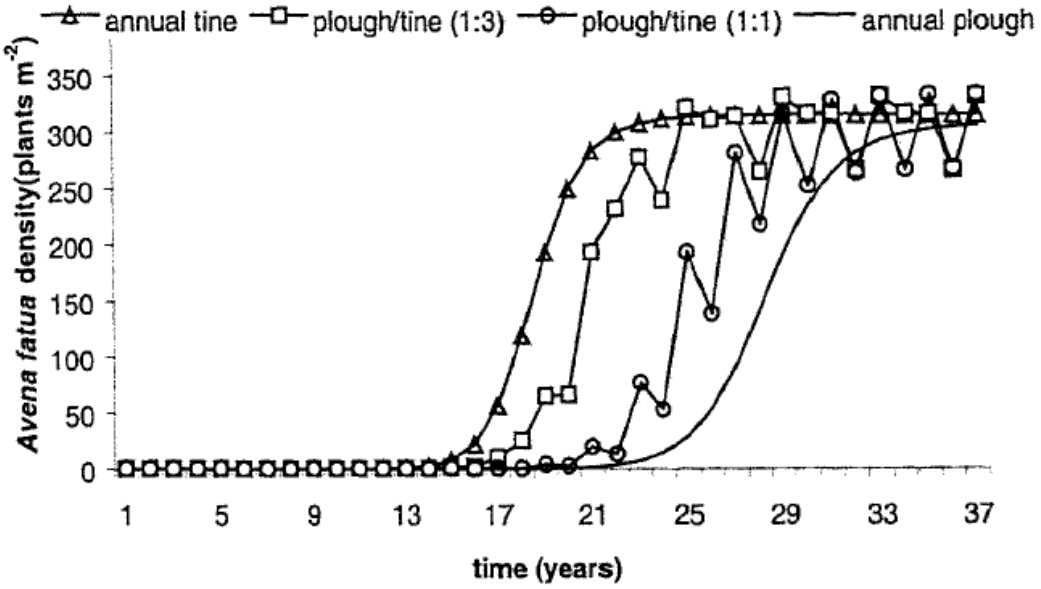


Fig. 6 Effect of different cultivation regimes on the build-up of wild oat *(Avena fatua).* Aryloxyphenoxypropionate (AOPP) and cyclohexanedione (CHD) herbicides are applied each year achieving 90% mortality of susceptible plants. The starting seed bank consists of 100 newly shed seeds rn-5 there is a mutation rare of 10 per generation to resistance; 0.293 of ovules are cross-pollinated.

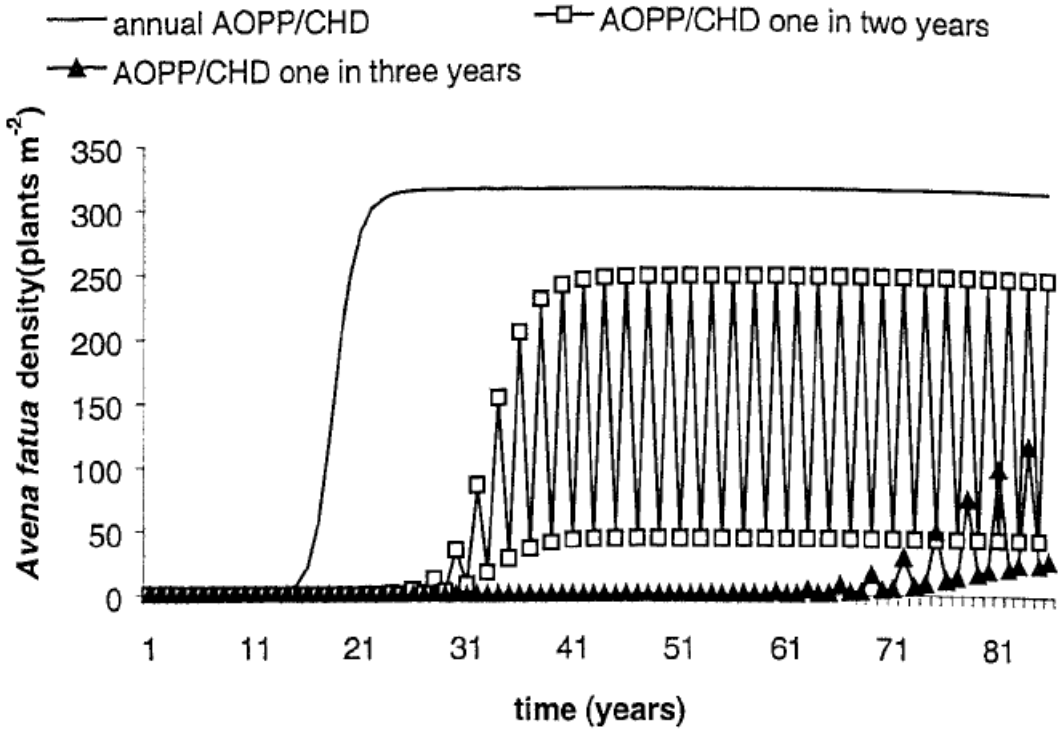


Fig. 7 Effect of continuous application of aryxophenosyproprionate AOPP) and cyclohexanedione (CHD) herbicides vs. rotation of two or three modes of action on the build-up of wild oat *(Avena fatua)* herbicides causing 90% mortality (with the exception of AOPP/CHD herbicides, which do not kill resistant plants). The starting seed bank consists of 100 newly shed seeds m^{-2}; there is a mutation rate of $1O^{-6}$ per generation to resistance; 0.2% of ovules are cross-pollinated.

In their model, herbicides act to reduce the seed bank and then maintain populations at 0.02 to 0.07 plants m^2 (200 to 700 plants per ha) in the years preceding development of field resistance. In real populations of this density, resistance may be increasing from a low initial frequency for many years before serious infestations are observed. An early warning of resistance evolution could be gained if seed samples from occasional rogued plants are screened. *One also can learn that wild oats are very difficult to control as weeds and everything should be avoided to produce wild oat populations with unwelcome genes or transgenes.*

(Gonzalez-Andujar & Fernandezquintanilla, 1993) did similar studies in Spain, and found, *that results to fight wild oats were much better without a fallow year inbetween*: A bioeconomic model is described and used to investigate the agronomic and economic consequences of using a range of management strategies for the control of winter wild oats (Avena sterilis L.) in cereal cropping systems representative of central Spain. The results of simulations indicated that growing winter wheat continuously with the annual application of herbicides may be the optimum strategy, resulting in acceptable wild oat populations and maximum economic benefits. However, the practice of wheat monoculture was only a valid option as long as herbicides were applied annually: spraying herbicides in alternate years failed to control wild oats adequately and resulted in major economic losses. The rotation of wheat with a fallow year, with no herbicides applied in either of the two years, may be a satisfactory low-cost alternative when wild oat infestation levels are low, but it is not valid when infestation levels are high. The strategy that combines the use of a fallow year with herbicide application in the wheat year resulted in optimum wild oat control and moderate profitability under all conditions. However, the net returns obtained were substantially lower than in the continuous wheat plus herbicide strategy. The sensitivity of the model to variation in various key parameters was tested: wheat yield level and fixed costs were the two parameters that had the largest effect on model output. In general, the effect of changing parameter values was more pronounced in continuous wheat systems than in wheat-fallow rotations.

In a further model evaluation, (Gonzalez-Andujar & Perry, 1995) came to similar conclusions:

- A metapopulation neighbourhood model of the seed bank of an annual plant, that included the effects of heterogeneity in space and time, of stochastic local extinction and of dispersal, was modified using data reported previously, to examine control of the arable weed *Avena sterilis.*
- In the absence of herbicide, for spatially homogeneous environments, few differences were found in the modelled mean predicted population for two levels of dispersal (strong and moderate), although the rate of spread and the variance of the number of seeds per cell were greater for the higher level of dispersal. For

spatially heterogeneous environments, with strong dispersal, an increase of the spatial scale of patchiness increased the variance, whereas moderate dispersal had the opposite effect. The introduction of temporal heterogeneity did not affect the results greatly; nor did the inclusion of variation in the fecundity parameter.

- With the introduction of a herbicide with spatially variable efficacy, the modelled metapopulation in all cases declined exponentially and became globally extinct in approximately 20 years; strongly dispersed populations with large-scale spatial heterogeneity were slightly more persistent. However, in all cases, it was usually possible to decrease the population within the model to acceptable levels (<I0 seedlings m-2) within a period of between **3** and 5 years. Spatial variability was considerable and extreme patch persistence was occasionally observed.

(Murray et al., 1995) documented the fate of another herbicide tolerance in wild oats: Resistance to fenoxaprop-P and other aryloxyphenoxypropionate and cyclohexanedione herbicides in the wild oat population, UM1, is controlled by a single, partially dominant, nuclear gene, In arriving at this conclusion, parents, F-1 hybrids, and F-2 plants derived from reciprocal crosses between UM1 and a susceptible wild oat line, UM5, were treated with fenoxaprop-P over a wide range of dosages, Based on these experiments, a dosage of 400 g al ha(-1) fenoxaprop-P was selected to discriminate between three response types, At this dosage, susceptible plants were killed and resistant plants were unaffected, whereas plants characterized as intermediate in response were injured but recovered, Treated F-2 plants segregated in a 1:2:1 (R, I, S) ratio, indicative of single nuclear gene inheritance, This was confirmed by selfing F-2 plants and screening several F-3 families, Families derived from intermediate F-2 plants segregated for the three characteristic response types, whereas those derived from resistant F-2 plants were uniformly resistant, Chi-square analysis indicated the F-2 Segregation ratios fit those expected for a single partially dominant nuclear gene system, In addition, F-2 populations from both crosses were screened with a mixture of fenoxaprop-P and sethoxydim, The dosages of both herbicides (150 g al ha(-1) fenoxaprop-P and 100 g ha(-1) sethoxydim) were sufficient to control only susceptible plants. Treated F-2 populations segregated in a 3:1 (R:S) pattern, thereby confirming that resistance to the two chemically unrelated herbicides results from the same gene alteration.

In some further field experiments the above results were confirmed by (Murray et al., 2002):
Two separate field experiments were conducted to quantify the degree of plant-toplant outcrossing and pollen-mediated gene flow (PMGF) in wild oat. The purpose of the study was to determine rhe extent to which pollen movement could contribute to the spread of herbicide resistance in this species. In both experiments, an acetyl-CoA carboxylase inhibitor-resistant R wild oat genotype (UM1) was used as the pollen donor and a susceptible (S) genotype (UM5) was used as the pollen receptor. Hybrid progeny resulting from a cross between UM1 and UM5 were identified using the herbicide resistance trait as a marker. In the plant-to-plant outcrossing experiment, single UM5 plants were closely surrounded by 20 homozygous R UM1 plants in hills. By screening seed from the S parent for resistance, outcrossing was determined to range from 0 to 12.3%, with a mean of 5.2% over 10 hills. In the PMGF experiment, single homozygous R UMI plants were surrounded by UM5 plants arranged in a hexagonal pattern at low and high densities (total of 19 and 37 wild oat plants m^{-2}), growing within spring wheat

and flax crops. In the wheat crop, mean wild oat outcrossing was 0.08 and 0.05% at low and high densities, respectively. In the less competitive flax, corresponding outcrossing values were 0.07 and 0.16% at low and high densities, respectively. Distance from the ~ollenso urce was a significant factor only for the high-density planting arrangement in flax. Up to 77 R hybrid seeds were recovered from 6 m2 in the PMGF experiment, indicating that PMGF contributes to the evolution of resistance in wild oat populations. However, the contribution of pollen movement ro resistance evolution and the spread of resistance in wild oat populations would be relatively small when compared with R seed production and dispersal from a resistant plant.

(Page et al., 2006): The spatial and temporal pattern of wild oat emergence in eastern Washington is affected by the steep, rolling hills that dominate this landscape. The objective of this study was to assess the impact of landscape position and crop residue on the emergence phenology of wild oat. Emergence of a natural wild oat infestation was characterized over two growing seasons (2003 and 2004), at two wheat residue levels (0 and 500 g m(-2)), and at five landscape positions differing in slope, aspect, and elevation in a no-till winter wheat field. Wild oat emerged 1 to 2 wk earlier at south-facing landscape positions than at north-facing landscape positions. Crop residue delayed wild oat emergence by 7 to 13 d relative to bare soil at south-facing positions in 2003 and had a reduced effect on emergence at north-facing landscape positions. Therefore, preserving surface residues tended to synchronize emergence across the landscape and may facilitate better timing of weed control where residue is present. Emergence of wild oat was modeled as a function of thermal time adjusted by water potential using a Weibull function. Temperature explained more variation in the model than water potential. This model explained much of the variability in wild oat emergence among landscape positions over these 2 yr and may be useful as a tool to predict the timing of wild oat emergence. Results also indicate that site-specific modeling is a plausible approach to improving prediction of weed seedling emergence.

A study was conducted by (Beckie et al., 2005) at a 64-ha site in western Canada to determine how preventing seed shed from herbicide-resistant wild oat affects patch expansion over a 6-yr period. Seed shed was prevented in two patches and allowed to occur in two patches (nontreated controls). Annual patch expansion was determined by seed bank sampling and mapping. Crop management practices were performed by the grower. Area of treated patches increased by 35% over the 6-yr period, whereas nontreated patches increased by 330%. Patch expansion was attributed mainly to natural seed dispersal (nontreated) or seed movement by equipment at time of seeding (nontreated and treated). Extensive seed shed from plants in nontreated patches before harvest or control of resistant plants by alternative herbicides minimized seed movement by the combine harvester. Although both treated and nontreated patches were relatively stable over time in this cropping system, preventing seed production and shed in herbicide-resistant wild oat patches can markedly slow the rate of patch expansion.

Final remarks: the problem with transgenic oats to be released in the field is not primarily the outcrossing dynamics, but the fact that the cultivated oats have some really nasty weeds as close relatives, which will be very difficult to control.

Bibliography of wild oats scientific literature from the Web of Sciences:
http://www.botanischergarten.ch/EPOBIO-Avena/Bibliography-wild-oats-20070121.pdf

Gene Flow Assessment for Avena

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) southern Europe yes, northern Europe no	1
CPW2	Is there a probability that the crop will flower and produc 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
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
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
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 by 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

Strand	Question	Score
CSF	**Propensity for successful seed- and tiller mediated gene flow from commercial crop to feral**	
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 a biodiversity threat ? (0/1)	1
CGC3	Does crop produce volunteers and/or seed deposits and pos 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)	4 (J)
CMG2	Mitigation with molecular safety measures apomixis AP, cytoplasmatic sterility CS, tandem construct TA, gene switching GS AP, TA, GS, (0/1)	AP, CS, TA, GS, 1

Beta vulgaris, Beet

1. Taxonomy

Latin : Beta vulgaris subspecies vulgaris
French : Betterave
German : Mangold, Krautstiele, Bete, Rote Rübe, Betarübe
English : Beet

Beet belongs to the genus Beta, the family Chenopodiaceae and the species Beta vulgaris. Beta vulgaris comprises several cultivated forms of B. vulgaris subsp. vulgaris. Cultivars include leaf beet (var. cicla) and root beet (var. esculenta).
Beta sect. Beta encompasses closely related wild, weedy, and cultivated forms of which more than 4350 unique accessions are maintained in seed collections. 250 samples have been classified as leaf beets. Since Beta germplasm is held by various genebanks in the world an internationally accepted classification system should exist, capable to transmit reliable information on Beta genetic resources. Such a consistent classification system for Beta sect. Beta is unfortunately not available, deplored by (Frese, 1991), and still today the situation is not much better. However, the accurate classification of Beta accessions would be a fundamental prerequisite for a purposeful choice of germplasm from collections (Frese, 1991), citing other sources.

Fig. 8 Biogeography of Beta vulgaris: Green: Area of cultivation, red: area of origin http://www.mpiz-koeln.mpg.de/oeffentlichkeitsarbeit/kulturpflanzen/Nutzpflanzen/Mangold/index.html

1.1. Classification of beets and problems with cultivars

(Frese, 2003) summarizes the present view on the complex taxonomy of Beta as follows: Though there is a long history of taxonomic research in Beta, no fully consolidated taxonomy of the genus exists. Before 1999, collectors of Beta germplasm had to deal with unsettled taxonomic problems as it is expressed by the 142 synonyms listed by the Mansfeld database (http://ma sfeld.ipk-gatersleben.de/mansfeld/), until two revisions, namely that of Beta section Corollinae (Buttler, 1977) and of Beta section Beta (Letschert, 1993) were published. Both contributions improved our knowledge of the taxonomic structure of the genus. (Buttler, 1977) published the correct names of the four sections as shown in Table 1. Beta section Beta is composed of three species: B. vulgaris, B. macrocarpa and B. patula. B. vulgaris is further divided into two wild subspecies and the cultivated subsp. vulgaris with its four cultivar-groups (Lange et al., 1999; Letschert, 1993). (Lange et al., 1999) argued that none of the morphological or cytological characters are suited to unambiguously delineate taxa within the cultivated beet. All characters used by (Helm, 1957) to distinguish 19 different types of cultivated taxa are subject to continuous variation and will not allow to clearly discern cultivated forms. (Lange et al., 1999), therefore, suggested to apply an open classification system as proposed also by (Hetterscheid et al., 1999; Hetterscheid et al., 1996). The introduction of the “culton” (plural “culta”) as taxonomic entity for cultivated beets allowed the formation of large and unambiguous cultivar-groups which can be internationally understood and can be easily handled by users of germplasm who are not familiar with the International Code of Botanical Nomenclature (ICBN) and Latin names. Consequently, the World Beta Network (WBN) recommended the use of the nomenclature as shown in the table below. The introduction of cultivar-groups has simplified the nomenclature and is welcomed as a useful pragmatic approach by beet breeders and other scientists dealing with applied research.

A pictorial summary of the cultonomy view erected by various authors like (Hetterscheid et al., 1999) and (Lange et al., 1999):

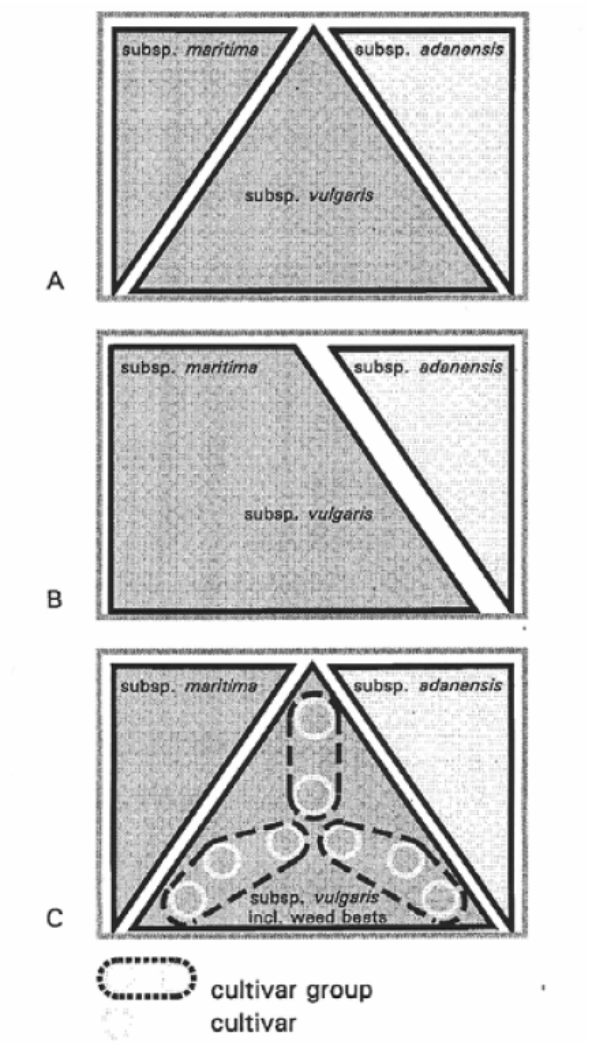


Fig. 9 History of the classification of Beta vulgaris L. A: botanical classification according to (Letschert, 1993). B: another way of botanical classification, without consideration of the nature of wild versus weed populations. C: botanical classification and oben classification with application of the subspecies to distinguish wild and weed pupulations (modified after (De Wet, 1981). From (Lange et al., 1999)

A useful table in beet taxonomy has been summarized by (Frese, 2003)

Primary genepool	**Section *Beta* (syn. *Vulgares* Ulbrich)** *B. vulgaris* L. subsp. *vulgaris* (cultivated beets) Leaf Beet Group Garden Beet Group Fodder Beet Group Sugar Beet Group subsp. *maritima* (L.) Arcang. subsp. *adanensis* (Pamuk.) Ford-Lloyd and Will. *B. macrocarpa* Guss. *B. patula* Ait.
Secondary genepool	**Section *Corollinae* Ulbrich** • Base species *B. corolliflora* Zosimovich *B. macrorhiza* Steven *B. lomatogona* Fisch. and Meyer • Hybrid species *B. intermedia* Bunge *B. trigyna* Waldst. and Kit. **Section *Nanae* Ulbrich** ***B. nana* Boiss. *and* Heldr.**
Tertiary genepool	**Section *Procumbentes* Ulbrich (syn. *Patellares*)** *B. procumbens* Smith *B. webbiana* Moq. *B. patellaris* Moq.

Fig. 10 Overview of taxonomy of Section Beta (synonymous to Vulgares Ulbrich), from (Frese, 2003) and as described in detail in (Frese et al., 2001b).

However, there are also disadvantages. A taxonomic name like "*Beta* L. *vulgaris* subsp. vulgaris convar. vulgaris provar. flavescens Lam. and DC. f. rhodopleura (Alef.) Helm" readily transfers the information that this germplasm is a red coloured leaf beet with broad petioles. With the replacement of this name by the culton "Leaf Beet Group" this descriptive information linked with the name is lost unless data on petiole width, length, colour etc. have been recorded in an evaluation database. As long as descriptive databases are incomplete it is essential to document the synonyms of accepted names in parallel.
And further on from (Frese, 2003):
(Lange et al., 1999) mentioned another problem. (Letschert, 1993) treated wild species of section Beta, only, and explicitly did not deal with the weedy and cultivated material. As a result, there is no formal link between the wild and cultivated classification system and no possibility to classify weedy types. It is therefore difficult to develop a determination key for the whole genus as noticed by (Frese et al., 2001a). In addition to

taxonomic problems of Beta section Beta, more research is required to consolidate the taxonomy of section Corollinae. (Buttler, 1977) in his thorough revision of section Corollinae could not deal with the hybrid complex in detail nor could he validate the existence of a B. foliosa in Turkey. Section Procumbentes might also need a revision since there are indications that B. webbiana and B. procumbens are closely related if not even identical species (Wagner et al., 1989). Furthermore, there are reasons to assume that the section *Procumbentes* does not at all belong to the genus *Beta* but to a separate genus ((Jung et al., 1993; Williams et al., 1976). Nevertheless, major users of germplasm holdings are satisfied with the taxonomic system presented in Fig. 1 as it transfers all the information they need.

But it would be illusionary to think, that the cultonomy will be an easy approach, the culton diversity and variety is enormous, and with more modern breeding attempts the 'evolution' of such culta will show increasing pace. Already with the classic cultons the attempt to discriminate the different traits is not easy, as shown by a figure from (Helm, 1957):

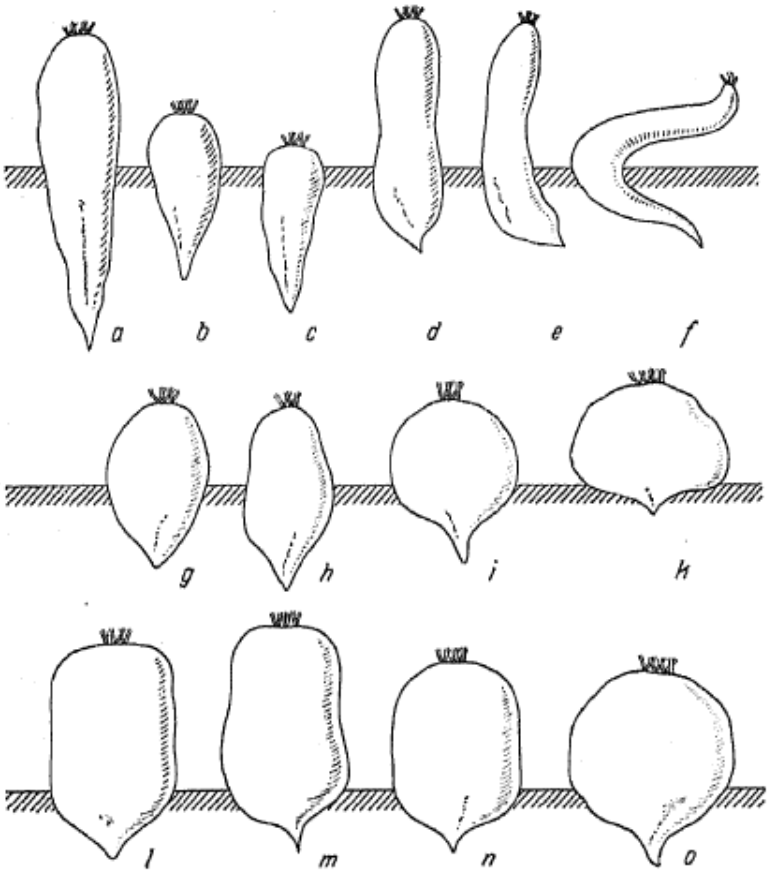


Fig. 11 Scheme of the best known pro-varieties crassa and altissima Döll. a – c: pile-shaped 'Veni-Vidi-Vici', 'Halbzuckerrübe', 'Kleinwanzlebener Zuckerrübe'; d – e: bottle-shaped 'Frankes Rekord', f: shaped like a cows horn 'Weisse Kuhhorn', g – h: olive-shaped 'Ovana', 'Barres', i – k: spheric 'Umstätter' 'Oberdörfer', l – n: barrel-shaped 'Eckendorfer' 'Criewener' 'Kirsches Ideal', o: cask-shaped 'Altenburger Tonne'. An extensive table see p. 215, From (Helm, 1957).

This figure manifests the difficulty to distinguish the traits i: ‘Umstätter’ from o: ‘Altenburger Tonne’ with means of shape characters.

The real complexity of shape based genetics has been revealed by (Baranski et al., 2001), one figure from his paper reveals this clearly:

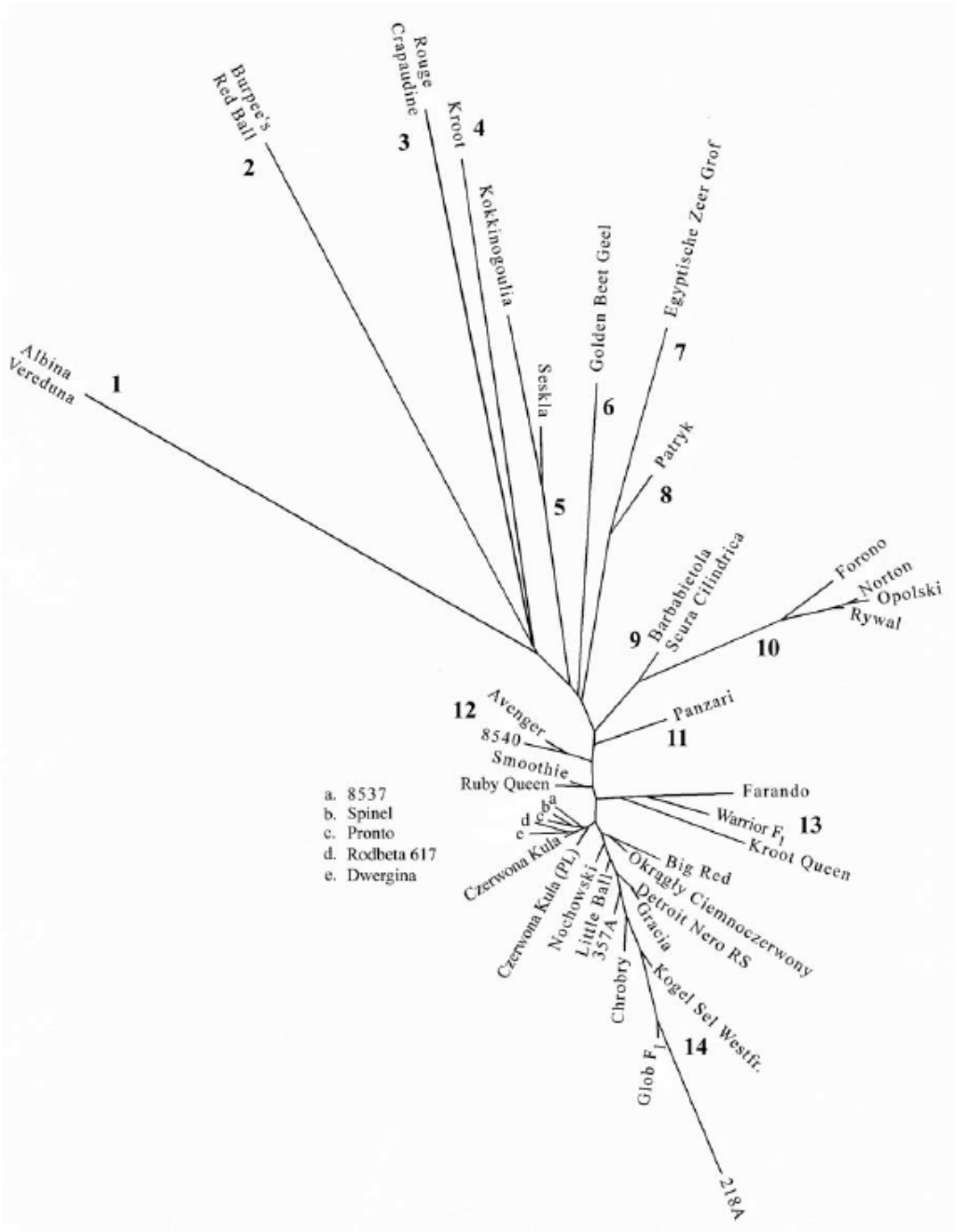


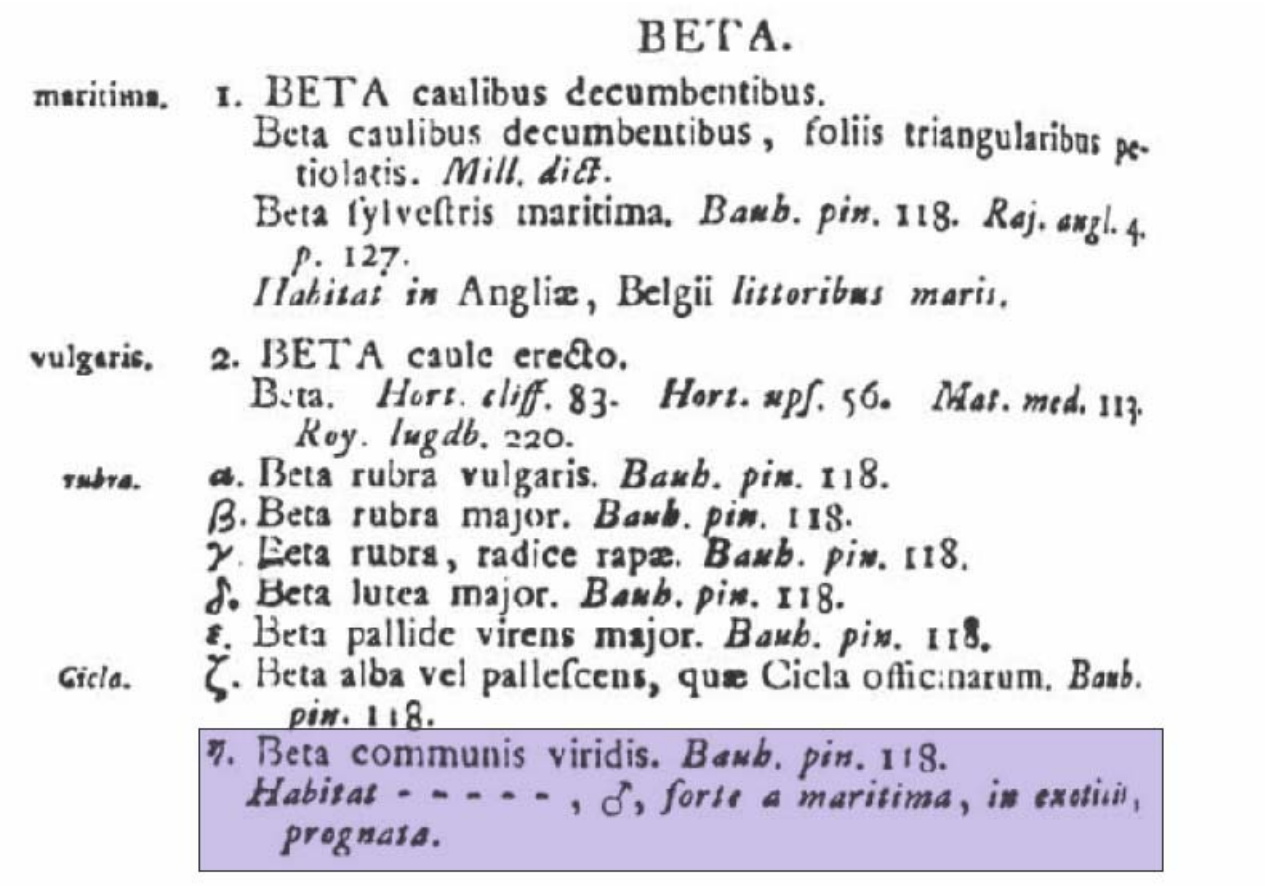
Fig. 12 Diversity with regard to root morphology of 40 accessions of *Beta vulgaris* subsp. *vulgaris* (Garden Beet Group). From (Baranski et al., 2001), who calculated a similar diagram for the chemical components with different branches,

Often the roots within a particular accession were divided into different classes with regard to a given trait. In some extreme cases, roots in one accession belonged to all 9 classes, e.g. skin roughness of cultivar ‘Warrior F1’. Assessment of the complex data table using correspondence analysis allowed to reduce the 39 root morphological trait classes evaluated to a few principal components. The first three components accounted for 62% of variation. They were mainly correlated to skin and flesh colour, and in some extend to root shape. These three components allowed to distinguish singular accessions like ‘Albina Vereduna’, ‘Golden Beet Geel’, ‘Burpee’s Red Ball’, and Rouge Crapaudine with regard to pigmentation, as well as three groups of cultivars with a narrow elliptic, circular and narrow oblong root shape. All of the accessions were well represented in a 10-dimensional space, where the axes accounted for 97% of the observed variation.
The calculated distances between accessions enabled to visualise the structure of their diversity in an unrooted tree (Figure 1). The core of the tree contained 17 accessions, which were similar in root morphology.
They were characterised by circular root shape, dark red skin with a low level of roughness, dark red flesh, and dark red or non-visible rings. The most distinct accessions were classified to 14 separate groups named by numbers. Nine of these groups contained only a single accession, while the other five consisted of 2 to 4 accessions.

The conclusion: Modern classification must be based on the methods combined of numerical taxonomy and thorough genetic molecular analysis.

1.2. Origin of beets

The cultivated form originates from the Mediterranean area, in the Near East. All cultivated beets may have originated from *B. maritima*, as already (Linnaeus, 1753) knew, but did not admit it officially, and indeed on p. 222 of his classic book he gave a cryptic hint on his own evolutionary view of species origins, which he had to hide at that time of the ruling creationism in order not to provoke the church ‘unnecessarily’.



BETA.

maritima. 1. BETA caulibus decumbentibus.
Beta caulibus decumbentibus, foliis triangularibus petiolatis. *Mill. dict.*
Beta ſylveſtris maritima. *Bauh. pin.* 118. *Raj. angl.* 4. *p.* 127.
Habitat in Angliæ, Belgii *littoribus maris.*

vulgaris. 2. BETA caule erecto.
Beta. *Hort. cliff.* 83. *Hort. upſ.* 56. *Mat. med.* 113. *Roy. lugdb.* 220.

rubra. α. Beta rubra vulgaris. *Bauh. pin.* 118.
β. Beta rubra major. *Bauh. pin.* 118.
γ. Beta rubra, radice rapæ. *Bauh. pin.* 118.
δ. Beta lutea major. *Bauh. pin.* 118.
ε. Beta pallide virens major. *Bauh. pin.* 118.

Cicla. ζ. Beta alba vel palleſcens, quæ Cicla officinarum. *Bauh. pin.* 118.

η. Beta communis viridis. *Bauh. pin.* 118.
Habitat - - - - - , ♂, *forte a maritima, in exotici prognata.*

Fig. 13 bottom of page 222 of Linaeuss Species Plantarum, with his cryptic hint on the evolution of Beta vulgaris, from (Linnaeus, 1753)

Beta vulgaris L. ssp. maritima, wild sea beet, is regarded as the mother species of the Beta beets (fodder beet, sugar beet, beetroot, yellow beet, Swiss chard). (OECD, 2001a). It is indigenous to European coastal regions, particularly the Mediterranean. Beet spinach, convar. cicla, has been cultivated in the Mediterranean region since 2000 B.C. In Europe B. vulgaris species with distinctly swollen roots were cultivated in the Middle Ages. Central European types are presumed to be descended from those used in Arabian horticulture in Spain. These plants were taken to the Netherlands, where they were cultivated beginning in 1500, and then to the Palatinate region, later spreading throughout Germany as “Burgundy beet”. During the sixteenth and seventeenth centuries, red and yellow beets became increasingly common as salad vegetables. Fodder beet cultivation only began to increase during the course of the eighteenth century. The crop was introduced into the USA in 1800 where it became known as a garden beet. Sugar beet was introduced to North America around 1830 and to South America circa 1850 (Mansfeld, 1986).

(Bartsch & Ellstrand, 1999) present a dendrogram showing the relationships between cultivated and wild beets:

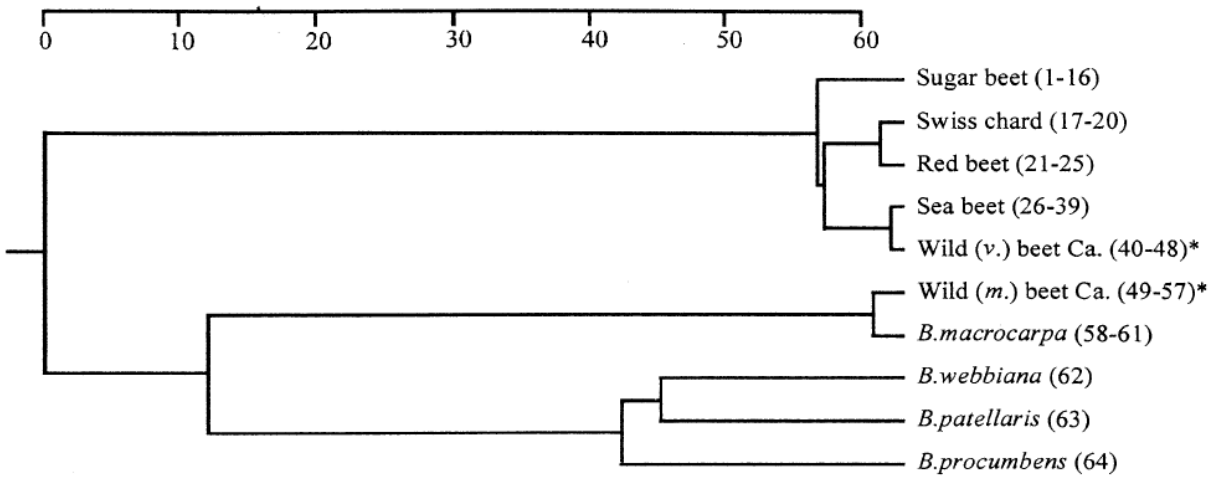


Fig. 14 UPGMA dendrogram of systematic relationships among ten major groups (with accession number) of wild and cultivated beet based on Nei's (1978) genetic distances derived from allele frequencies at 13 polymorphic allozyme loci. From (Bartsch & Ellstrand, 1999)

To evaluate relationships among groups of accessions, we constructed a UPGMA dendrogram based on (Nei, 1978) genetic distances (Fig. 3). According to this pattern, the Californian wild beets have major affinities to both B. vulgaris and B. macrocarpa. In the tree, sea beet clustered with the wild v.-type beet, and the wild m.-type clustered with Old World accessions of B. macrocarpa. By incorporating the three different outgroup species, the unique nature of the two distinct wild Beta species in California is clearly apparent. The genetic distance obtained for B. vulgaris and B. macrocarpa is nearly as great as the distance of each of them to the outgroups of B. procumbens, B. webbiana, and B. patellaris (all of which are cross-incompatible with B. macrocarpa and B.vulgaris). It is remarkable that, despite their substantial distances, hybridization between B. vulgaris and B. macrocarpa is still possible, as both allozyme markers in this study demonstrated and prior literature (Abe et al., 1986; Coons, 1975) has suggested. In summary, we found strong evidence for the classification of B. macrocarpa as a separate species from B. vulgaris. Although the two species are cross-compatible, they are clearly differentiated at the molecular level. In summary, (Bartsch & Ellstrand, 1999) found strong evidence for the classification of *B. macrocarpa* as a separate species from *B.vulgaris.* Although the two species are cross-compatible, they are clearly differentiated at the molecular level.

According to (Boutin et al., 1987) mitochondria1 and chloroplast DNAs from Beta maritima and cultivated Beta vulgaris plants were compared in our study. No variability of chloroplast DNA could be detected between the two taxa. Fertile plants of Beta maritima and Beta vulgaris gave closely similar restriction patterns of mitochondria1 DNA. Beta maritima hermaphrodites from two different provenances present the same pattern and differed from *Beta vulgaris* mtDNA only by three bands. The interesting results of the present study of the Canche population demonstrate a novel cytoplasmic male sterility system which differs from that described by Owen.

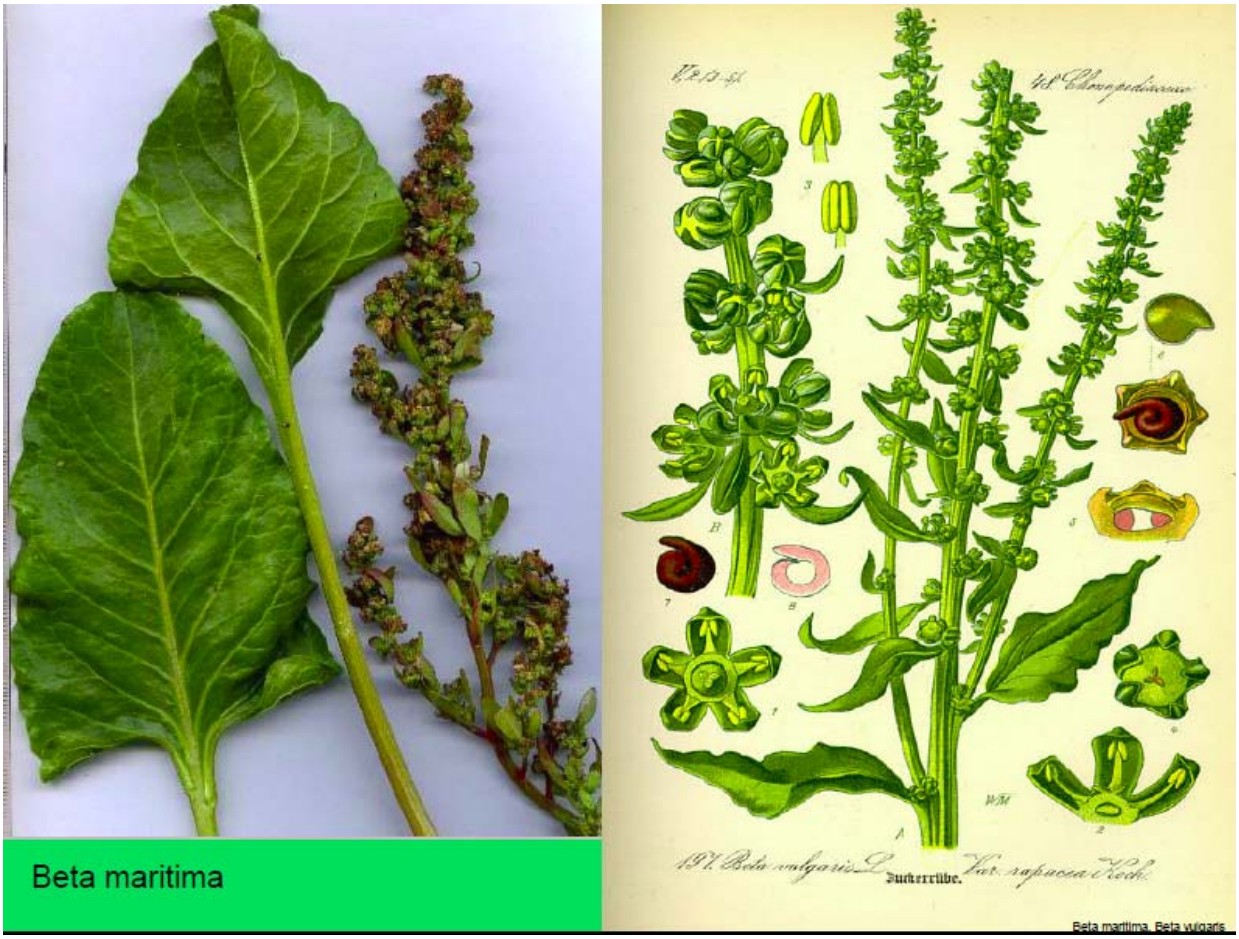


Fig. 15 Beta maritima left: http://perso.orange.fr/argaud/botanique/beta_vulgaris_maritima.html right: from Prof. Dr. Otto Wilhelm Thomé *Flora von Deutschland, Österreich und der Schweiz* 1885, Gera, Germany, downloadable from Wikimedia. http://commons.wikimedia.org/wiki/Image:Illustration_Beta_vulgaris_var._rapacea0.jpg

Beet is an annual, biennial or perennial plant. Crops are essentially biennial and are grown for the swollen root that it develops at the end of the first growing season.

A study by (Desplanque et al., 1999) demonstrated the intermediate position of weed beets between the cultivated and south-western ruderal inland gene pools of beets. Weed beets clearly appeared to be produced by accidental hybridization of cultivated lines and ruderal-beet pollen donors in the seed-production area. This result con"rms the previous study (Boudry et al., 1993; Boudry et al., 1994) which identified their maternal origin. Indeed, the genetic distances used by (Desplanque et al., 1999) are based on nuclear markers with a biparental transmission. This, in turn, makes it possible to infer the paternal contribution, which had been previously determined on the sole basis of the transmission of the bolting gene's B allele. Another interesting result that emerges from the present study is the high genetic diversity of weed beets despite their recent evolutionary history. Their high nuclear genetic diversity contrasts with the previously

found uniformity of mtDNA (Boudry et al., 1993; Boudry et al., 1994). This suggests that: (1) pollen flow from inland to cultivated beets is likely to be both frequent and recurrent, and (2) the transportation of crop-wild hybrids from the seed-production area to the sugar-beet fields in Northern France is also likely to be a recurrent phenomenon rather than the result of a single introgressive event followed by local expansion in sugar-beet fields. By using greenhouse studies on life-history traits, (Bartsch & Schmidt, 1997) suggested that a similar scenario occurs in northern Italy, the other important European seed-production area. Introgression between cultivated and wild beets could therefore be a general trend but one which has to be ascertained more accurately by fine-scale genetic analyses in crop-wild sympatric areas.

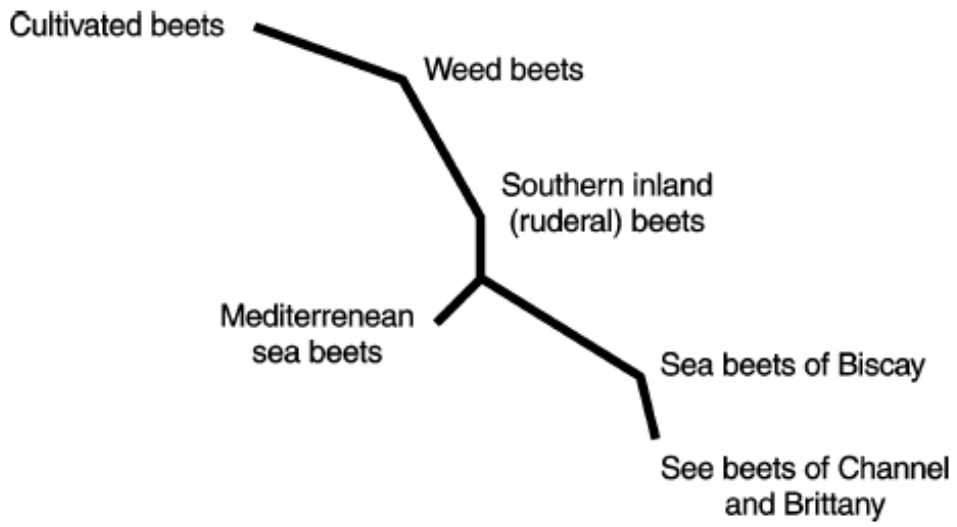


Fig. 16 Unrooted dendrogram inferred from Reynolds' genetic distance matrix between the di!erent forms of beet, based on six nuclear loci (Neighbor-Joining method). From (Desplanque et al., 1999)

The origin of south-western rural inland beets is less obvious. Their genetic diversity appears to be high, since one can find as many alleles in this group as among coastal forms. In addition, their closest relatives appeare to be the coastal beets, in particular those of the Mediterranean, but not the Atlantic beets despite the latter's closer geographic proximity. Genetic proximity is corroborated by the fact that many ruderal populations occur all along a geographic continuum, linking up the south-western seed-production area to the Mediterranean coastlines, while no populations were observed when moving towards the Atlantic coasts. Although these ruderal populations seem to be of wild origin, their position in the tree (Fig. 3) indicates an introgression with cultivated forms.

2. Reproduction biology

2.1. Flowering

Flowers of Beta vulgaris sspp vulgaris are located on the terminal portions of the main axis and on lateral branches subtended from this. Flowers are sessile and occur singly or in clusters of two to eight (Smith, 1980). Flowers are perfect and consists of a tricarpellate pistil surrounded by five stamens and a perianth of five narrow sepals (Smith, 1980). The flowers, solitary or in clusters of 2-8, are rarely selfpollinating (Free, 1970). The flower has a raised ovary with three or four secure stigmata. Three leaves are fused together into a single gynoecium to form the ovary. The seed arrangement is campylotropous.

Beets need a vernalisation in order to flower. However, a small proportion of plant flowers in their first year ("bolters") and may set seeds that persist in the soil. It is probably from this source and from volunteer or "groundkeeper" beets remaining after the harvest, that the population of annual beets that constitute the "weed beet" population arose (Hornsey, 1973a, b, 1975; Hornsey & Arnold, 1979). A second possible source of annual beets is the contamination of seed crops by pollination from annual wild beets (Evans & Weir, 1981; Hornsey & Arnold, 1979; Longden et al., 1974). This may have happened especially in southern Europe during the production of seed of triploid monogerm varieties, where the male-sterile diploids used as mother plants could have been especially susceptible to pollination by diploid plants rather than the intended tetraploids (Scott & Longden, 1970).

Flowering weed beets in sugar production areas have rapidly emerged as a serious problem since the early 1970's in Europe. This weed beet appears to be phenotypically different from volunteer sugar beet in that it produces more seed and in France this seed has been shown to usually not need a vernalization (Harding & Harris, 1994). The weedy form theoretically may have entirely evolved in parallel from bolters *in situ* in sugar producing areas - but more plausibly, molecular evidences suggest that weed beet originated from crosses in seed producing areas along the Mediterranean following by introgression from wild diploid species (Boudry et al., 1993).

Beet is basically a self-incompatible plant (Bruun et al., 1995; Lundqvist et al., 1973; Smith, 1980; Valdeyron, 1884) (the stigma is not fully mature when the flower opens). It is an allogamous species, pollinated by wind and occasionally by insects, the former being the most important. Some cross-pollinations are also done by thrips and syrphids (Free et al., 1975; Valdeyron, 1884) Wind-borne pollen can be distributed horizontally at least 4,500 m and has been observed at vertical distance of 5,000 m (Archimowitsch, 1949). But Gliddon (Harding & Harris, 1994) assumed that the pollen movement takes place to around 8 km. In commercial practice, plantings of related seed types are separate by distances of 1.6 km or more. Fields of unrelated seed types usually are separated by 3.2 km or more, depending on terrain and prevailing winds (Campbell & Mast, 1971).

2.2. Seed dispersal

Sugar beet possesses long-lived dormant seeds that can become a volunteer weeds in sugar beet fields (Hojland & Pedersen, 1994). They tend to germinate in the field 1-3 days later than planted sugar beet seeds (Hojland & Pedersen, 1994). Sugar beet seeds may remain in the soil for ten years or more and still retain some germination capacity (Brouwer et al., 1976; Lysgaard, 1991; OECD, 1993). It is generally accepted that six year-old multigerm and four year-old monogerm sugar beet seed exhibit the same germination level of 70%. Eight-year-old sugar beet seeds have been shown to germinate at a level of 59% in laboratory conditions. These germination percentages depend of the quality of the seeds and of the conditions of germination. Thus Beta vulgaris has the ability to generate a viable seed bank (Hojland & Pedersen, 1994). The seed-balls of Beta are resistant to salt water, and ocean currents can move propagules over relatively long distances. Above the high water line, strong winds distribute them over the shoreline, and sometimes even inland (Smart, 1992).
Since commercial sugar producing sugar beet is biennial and is harvested during the first year whilst still in the vegetative phase, sexual reproductive organs (floral parts) never develop. Varieties that tend to bolt in the first year of growth pose some problems and much effort has gone into developing currently cultivated varieties that limit bolting. When Beta vulgaris is planted for seed production, some seeds may remain on the field after harvesting the seed crop. Agricultural practices tend to limit those shoots.

2.3. Fertility, in- and outbreeding

Most of the sugarbeet grown since 1960s has been triploid. Triploids are produced by crossing tetraploid parents with diploid male sterile and are usually doubly sterile because of chromosome imbalance and cytoplasmically inherited male sterility. However, a small proportion of plants does produce aneuploid pollen some of which will give fertile progeny on crossing with diploids. Diploid varieties (2n=18) are now used more frequently, as they allow the production of true F1 and 3- and 4-way cross in breeding programs.

Certain inbred sugarbeet lines are reported to have developed apomixis and are thus able to reproduce without fertilisation (Bruun et al., 1995; Fang et al., 2004; Gao & Jung, 2002; Jassem, 1976; Jassem & Jassem, 1971; ReamonButtner et al., 1996).

A summary of breeding systems and seed yield of Beta vulgaris and relatives is given by (Frese, 2003)

Botanical name	Seed type	Prevailing breeding system*	Days from sowing to flowering	Average single plant seed yield in gr. (min–max)
B. vulgaris Leaf Beet Group	Normal	Outcrossing	up to 180	70 (15-170)
B. vulgaris Garden Beet Group	Normal	Outcrossing	up to 180	40 (15-70)
B. vulgaris Fodder Beet Group	Normal	Outcrossing	180	50 (15-70)
B. vulgaris Sugar Beet Group	Normal	Outcrossing	180	50 (15-70)
B. *vulgaris* subsp. *maritima*	Varying degrees of dormancy	Outcrossing	40-260	30 (4-110)
B. vulgaris subsp. *adanensis*	Varying degrees of dormancy	Inbreeding	40-60	20 (13-75)
B. macrocarpa	Varying degrees of dormancy	Inbreeding	40-60	20 (8-57)
B. patula	Normal	No records	40-60	12
B. corolliflora	Hard pericarp	Outcrossing	430	30 (25-50)
B. macrorhiza	Hard pericarp	Outcrossing	420	10 (1-21)
B. lomatogona	Hard pericarp	Outcrossing	450	5 (1-26)
B. intermedia	Hard pericarp	Apomictic	450	20 (15-25)
B. trigyna	Hard pericarp	Apomictic	430	30 (25-50)
B. nana	Hard pericarp	No records	No records	No records
B. procumbens	Hard pericarp	Outcrossing	60	20 (2-80)
B. webbiana	Hard pericarp	Outcrossing	60	20 (2-80)
B. patellaris	Hard pericarp	Inbreeding	60	20 (2-80)

*according to JASSEM (1992) and own observations

Fig. 17 Overview on breeding systems and seed yield of Beta vulgaris and its relatives, * according to (Jassem, 1992), from (Frese, 2003)

Beta vulgaris belongs to the section Vulgare with B. maritima, B. macrocarpa, B. patula and B. atriplicifolia that are the wild species of the cultivated beet (Valdeyron, 1884). All these species are cross-compatible (Smith, 1980). No evidence of interfertility has been found between the cultivated beet and the Caucasian beet (Beta trigyna), an introduced ornamental species.

The Beta vulgaris subspecies are relatively interfertile ; they are pollinated by both wind and insects, while being self-incompatible.

2.4. Resulting hybridisation

It is an old and persistent problem in assessing outcrossing impact that many studies do not distinguish between *potential* and *realized* outcrossing (gene flow, bolting and seed dispersal).
Sugar beet is normally triploid, produced by crossing tetraploid pollen parents with diploid male sterile genotypes. Such hybridisation cause chromosome imbalance and instability in hybrids that often results in both male and female sterility. However, it is assumed that bolters will hybridise with weed beets to some degree.

Sugar beet and sea beet (Beta maritima) are both protandrous, self-incompatible and gynodioecious. Male sterility is under the control of the cytoplasm with nuclear genes restoring male fertility (although cultivated and wild beet may have different nuclear and cytoplasmic components (Boutin et al., 1988; Boutin et al., 1987; Owen, 1945). They can hybridise freely and hybrids are spontaneously formed in the wild and in seed-production fields.
There is extensive evidence of hybridisation in the wild between, and introgression from wild beet to sugar beet.
Sugarbeet can hybridise with other wild beet species (B. procumbens, B. webbiana and B. patellaris) (Hojland & Pedersen, 1994). Strong hybridisation barriers exist between sugarbeet and *B.* vulgaris subsp. Macrocarpa (but still records exist) or subsp. patula.
More references to hybridization dynamics of Beta vulgaris in (Bartsch et al., 1999; Desplanque et al., 2002; Driessen et al., 2001; Lorenz et al., 1994; ReamonButtner et al., 1996; Schmidt et al., 1997; Viard et al., 2004; Viard et al., 2002)

2.5. Bolting

A special problem for beet cultivation is bolting. (Boudry et al., 1994) demonstrated, that annual habit which results in bolting is due to complete or partial absence of the vernalization requirement and can cause severe problems in the beet crop. The absolute vernalization requirement in beet is controlled by a major gene B (bolting), known to be linked to the gene R (red hypocotyl color), in linkage group I. (Boudry et al., 1994) studied segregation for the B and R genes in several beet progenies. Penetrance of the annual habit in Bb genotypes was affected by both environmental and genetic factors. The precise location in linkage group I of the major gene B was found by restriction fragment length polymorphism (RFLP) analysis in a back-cross progeny exhibiting partial penetrance of the annual habit. Evidence of pseudo-compatibility was found in the wild coastal beet (Beta vulgaris ssp maritima) used as the mother plant of the back-cross: the selfing rate was estimated as 7%. See more on bolting in chapters on hybridization and risk assessment.

4. Biosafety considerations

4.1. General remarks

Sugarbeet may itself become a weed through the remaining roots or crowns left in the field after harvest. These sources of flowers need to be eradicated by effective herbicide.

Even without hybridisation, the transgene may be able to persist in weed beets derived from bolters or volunteers and from seeds remaining viable for many years in the soil. Introgression of these plants or of the wild beet with the crop and selection under cultivation could produce annual weed beet, as in the past, containing the construct. Thus the escape of the transgene to a crop weed, and perhaps to a lesser extent to a weed of disturbed habitats, is entirely plausible. It is a realistic scenario to deal in risk assessment and risk management with transgenic feral populations. This possibly will be made less likely by retention of doubly sterile triploid varieties, decreased frequency of bolting and by producing seed in selected areas away from the coast in southern Europe. It will be made more likely to depend on the method of designing the nature of the finished transgenic variety and the extent to which it can be mixed by diploids.

Cultivated beet may possibly run wild but it is difficult to distinguish between cultivated beets and the wild beet. Beet is often found outside cultivation but there is no indication of such plants establishing in the wild (Frietema, 1996).
Transfer of the transgene from cultivated beet to sea beet is also possible (Bartsch et al., 1999).
The sugarbeet does not generally reproduce asexually, but some varieties have been developed for apomixis (Hojland & Pedersen, 1994). See also: (Fang et al., 2004; Gao & Jung, 2002; Jassem, 1976; Jassem & Jassem, 1971). In conclusion, aware of the difficult gene flow situation, see chapter 4.2., apomictic Beta should be a desirable option.

4.2. Gene flow studies

Gene flow studies have been carried through by (Desplanque et al., 2002)
The figure below demonstrates the complexity of gene flow possibilities.

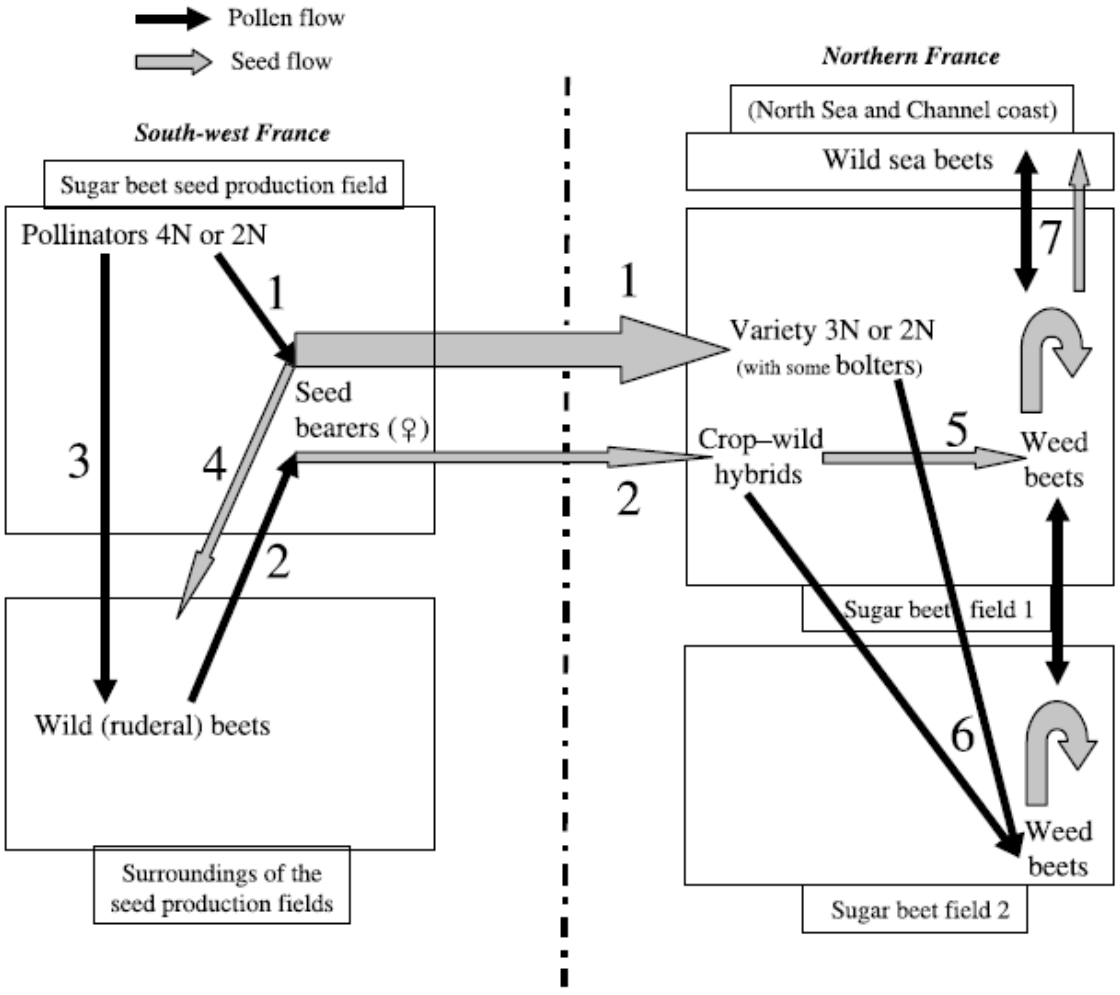


Fig. 18 A schematic presentation of the possibilities of gene flow by seeds and pollen in the sowing seed-production area (left) and in the sugar-production area (right). The seed bearers are male-sterile, the pollinator plants are hermaphrodite; all other plants can be both. The pollinator plants can be tetrapoid (4N) or diploid (2N), leading to triploid (3N) or diploid (2N) varieties, respectively; all other plants are usually diploid. From (Desplanque et al., 2002)

In a summary, the authors give a comprehensive view of gene flow in the seed production and sugar production areas in France, which are strictly separated:

1. Weed beets pose a serious problem for sugar beet Beta vulgaris crops. Traditionally, the only efficient method of weed control has been manual removal, but the introduction of transgenic herbicide-tolerant sugar beets may provide an alternative solution because non-tolerant weed beets can be destroyed by herbicide. The possibility that new, transgenic, weed beets may arise by gene flow between wild and crop plants was evaluated.

2. In a study area in northern France, weed beets were present in variable densities in sugar beet fields of up to 80 weed beet plants m^{-2}. Weed beets arise from a long-lived seed bank, with seeds germinating from depths of 5 cm or less. In addition, diploid F1 crop–wild hybrids and triploid variety bolters (individuals with a low vernalization requirement) were present in low densities in virtually all sugar beet fields. The authors found gene flow to be possible between all forms, illustrated by both overlapping flowering periods in the field and successful controlled cross-pollinations.

3. The F1 crop–wild hybrids result from pollination in the seed-production region by wild plants possessing the dominant bolting allele B for flowering without experiencing a period of cold. In the case of a transgene for herbicide tolerance incorporated into male-sterile seed-bearer plants, such hybrids will contain both the herbicide-tolerance and the bolting allele. Contamination of the fields by transgenic weed beets will be the result *unless bolters are removed manually.* The same will apply in the case of a cytoplasmically inherited transgene.

4. Incorporation of the transgene into the pollinator plants will prevent the immediate formation of transgenic weed beets. However, in sugar beet fields, variety bolters may successfully cross-pollinate with weed beets in neighbouring fields. The use of diploid pollinator plants instead of tetraploids will considerably enhance gene flow towards wild beets, and is not, therefore, an attractive option.

5. In conclusion, the appearance of transgenic weed beets is possible *but can best be* retarded if the transgene for herbicide tolerance is incorporated into the tetraploid pollinator breeding line.

4.3. Competition, fitness as an assessment factor

However, the research team of (Bartsch et al., 2001) demonstrated in field trials with transgenic sugar beet that the competition factor strongly influences the results. Transgenic beets with beet necrotic yellow vein virus (BNYVV) coat protein (*cp*), phosphinothricin-acetyl-transferase (*bar*), and neomycinphospho-transferase (*nptII*) genes were hand-crossed to Swiss chard. The resulting F1 plants and controls were grown at two different BNYVV infestation levels and three different competitive conditions with *Chenopodium album*. Transgenic hybrids had consistently higher biomass than controls under high background BNYVV infestation, ad consistently lower biomass than controls under low background infestation. The transgenic hybrids had a significantly lower rate of bolting than controls at all sites. Competition with Chenopodium album always had a strong negative influence on the performance of all genotypes. The authors conclude that ecological implications due to the introduction and spread of virus-resistant transgenic hybrids will be observed only in those feral Swiss chard and wild beet populations where
fitness is significantly influenced by high infestations of BNYVV.

Weed competition always had a strong negative influence on all genotypes (Fig. 5), but there was no statistically significant interaction between competition and genotype as well as between competition and virus infestation. Additionally, no three-way interaction was observed for genotype x competition x virus infestation.

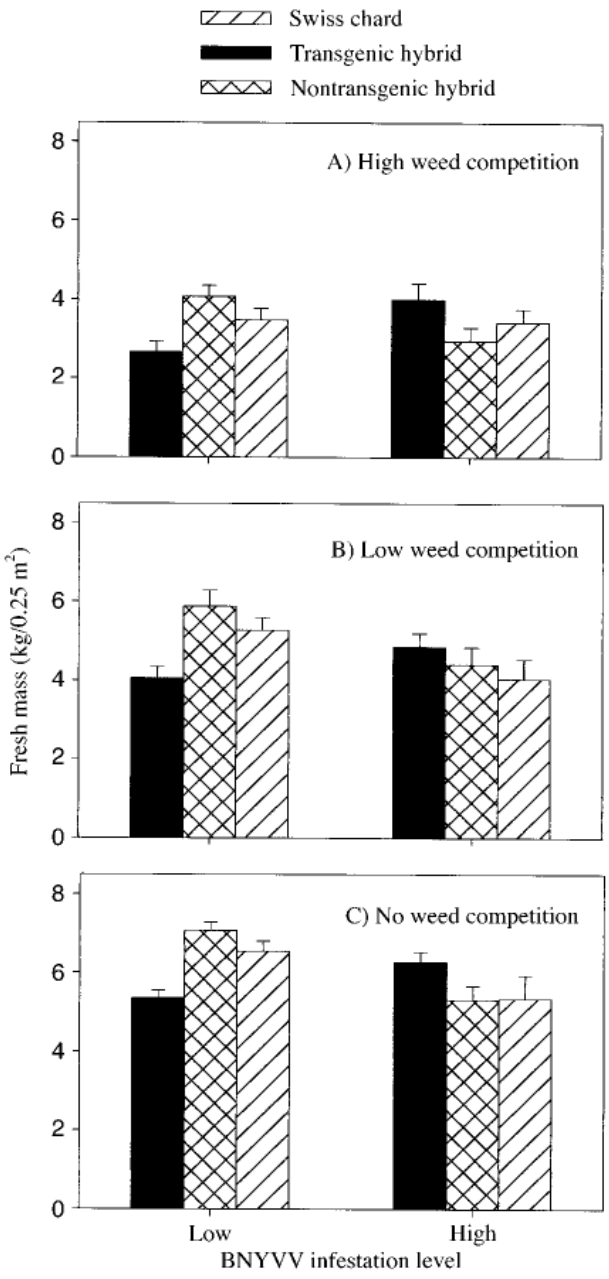


Fig. 19 Performance of transgenic and nontransgenic Beta vulgaris. The biomass production (1 1 SE) in the field test included a hybrid between transgenic sugar beet and Swiss chard, a nontransgenic hybrid, and the female Swiss chard parent. The plants were grown first in the greenhouse and then planted in three different competition densities with Chenopodium album (Fig. 1A–C) sites with either low or high BNYVV infestation. From (Bartsch et al., 2001)

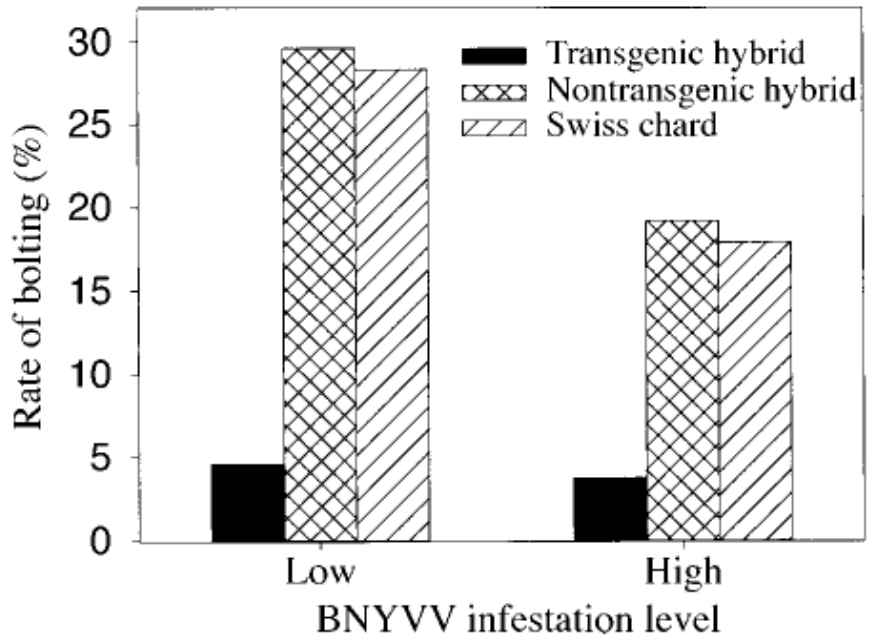


Fig. 20 Bolting rate of experimental plants in the study from (Bartsch et al., 2001)

In contrast to the hybridization rate not showing significant differences, the bolting rate of the transenic traits is significantly lower, probably due to pleiotropic effects: The transgenes cause a lower competitiveness as (Bergelson & Purrington, 1996) suggested with the term 'cost of resistance'. The unexpected phenotype demonstrates that genetic engineering may alter life histories in unintended ways (Linder and Schmitt 1995, Bergelson et al. 1998). For what ever reason, the bolting depression would probably reduce, but not eliminate, the risk of gene flow to wild relatives of cultivated plants. But as long as we have not more experience, we will have to anticipate that the bolting depression depends also of the nature of

4.4. Seed dispersal

(Arnaud et al., 2003), concluded, that although pollen usually represents a significant vector for the spread of genetically modified traits, the present results suggest: (i) that seed flow may have a deeper and longer impact in connecting wild and crop relatives within the complex *Beta*; and (ii) point out the key role of a long-lived seed bank, a factor often neglected. Sugarbeet seeds aged of 8 years can germinate till 59% in laboratory conditions.

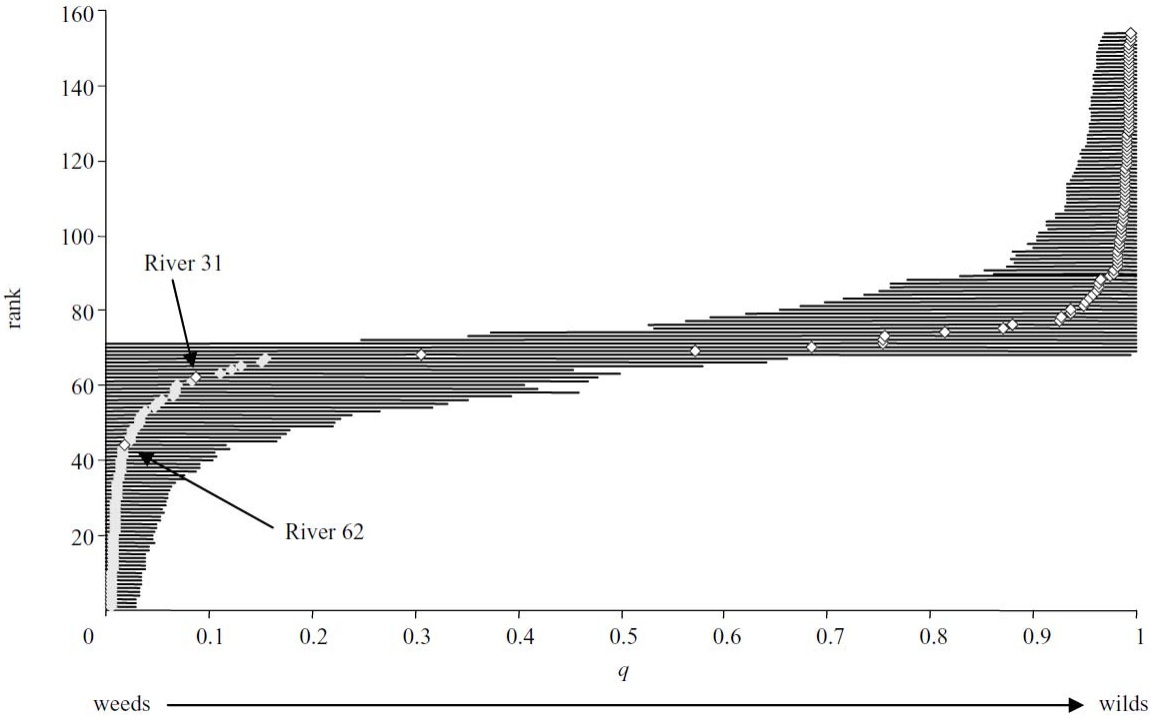


Fig. 21 Distribution of the mean individual admixture coefficients *q* estimated using Structure (Pritchard et al., 2000) without prior population information. In this analysis, *K* (the number of population contributing to the gene pool of all sampled individuals) is assumed to be 2. Individuals were ranked from lowest to highest *q*-values and ranks were plotted against *q*. A *q*-value of 1 denotes a wild individual, whereas 0 denotes weedy individuals. Also displayed are lines giving the 95% posterior probability intervals of *q* for each individual. The cytoplasmic status of individuals is represented by a grey diamond for a OwenCms cytoplasm and a white diamond for a non-OwenCms cytoplasm. From (Arnaud et al., 2003).

4.5. Mitigation of gene flow

4.5.1. Cytoplasmic Male Sterility (CMS)

In an extensive study, (Satoh et al., 2006) demonstrate the complexity of the location and function of the Owen Cytoplasmic Male Sterility (discovered by (Owen, 1945):
The mitochondrial genomes of normal fertile and male-sterile (Owen CMS) cytoplasms of sugar beet are highly rearranged relative to each other and dozens of inversional recombinations and other reshuZing events must be postulated to interconvert the two genomes. In this paper, a comparative analysis of the entire nucleotide sequences of the two genomes revealed that most of the inversional recombinations involved short repeats present at their endpoints.
Attention was also focused on the origin of the Owen CMS-unique mtDNA regions, which occupy 13.6% of the Owen genome and are absent from the normal mtDNA. BLAST search was performed to assign the sequences, and as a result, 7.6% of the unique regions showed significant homology to previously determined mitochondrial sequences, 17.9% to nuclear DNA, 4.6% to mitochondrial episomes, and 0.1% to plastid DNA. Southern blot analysis revealed that additional sequences of nuclear origin may be included within the unique regions. We also found that the copies of many short

repeat families are scattered throughout the unique regions. This suggests that, in addition to the incorporation of foreign DNAs, extensive duplication of short repetitive sequences and continued scrambling of mtDNA sequences may be implicated in the generation of the Owen CMS-unique regions.
This is illustrated in the below figure:

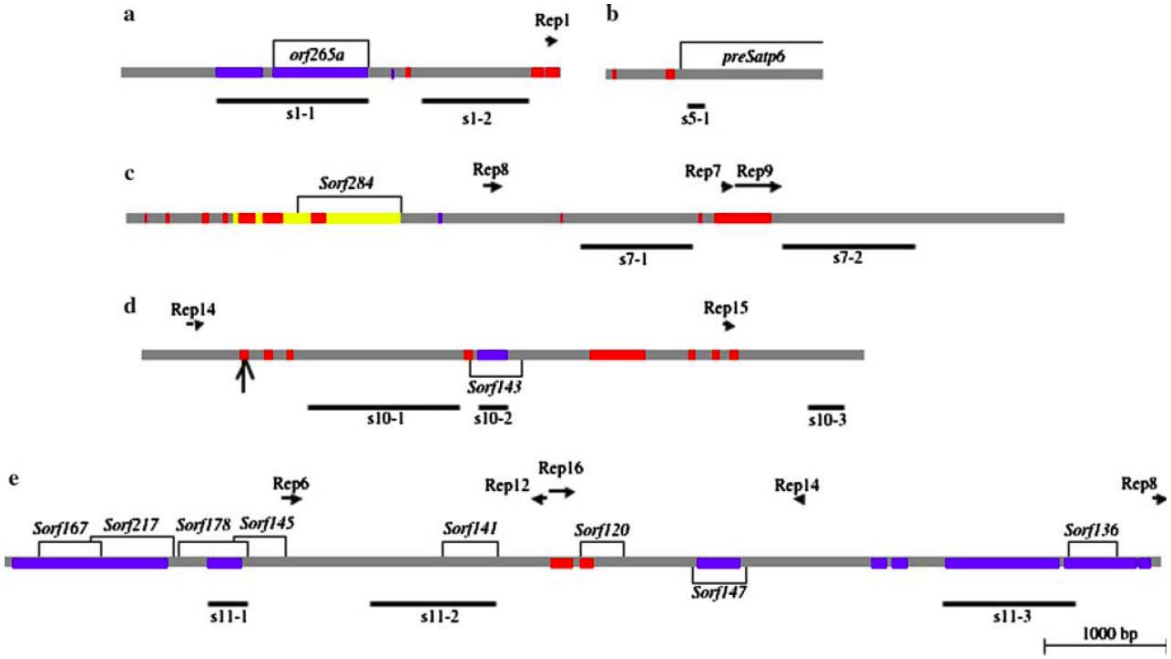


Fig. 22 Organization of Wve Owen CMS unique regions: s1 (a), s5 (b), s7 (c), s10 (d) and s11 (e). *Scale bar* is shown below. BLAST search revealed that the unique regions contain sequence segments homologous to nuclear DNA (shown in *blue*), previously characterized mtDNA sequences (*red*) and mitochondrial episome (*yellow*). The extent of ORFs is indicated by *open boxes*. Their direction is from left to right for those above lines and from right to left for those below lines. Probes for hybridization experiments are shown by *black horizontal bars*. Repeated sequence families are shown by *horizontal arrows*. A *vertical line* in d indicates a sequence segment homologous to *Arabidopsis* and rapeseed mtDNA but not to TK81-O mtDNA

The origin of Owen CMS is an interesting and open question. Bonavent et al. (1989) speculated that in the past, a cross occurred between a CMS plant of the old garden-beet cultivar ‘Crapaudine’ and a sugar-beet plant, and some individuals of the progeny were collected by Owen. The Owen cytoplasm was also reported to be rarely found in wild beet populations growing along the French coasts (Laporte et al., 2001). This raises the possibility that a fertile progenitor to the Owen cytoplasm and/or the derived sister cytoplasms may be discovered in garden-beet landraces or wild beet accessions. Such cytoplasms, if any, would be valuable materials to gain a better understanding of how and when the Owen mitochondrial genome was created. A lot has been published about CMS, some links are given in the bibliography: (Bonavent et al., 1989; Boutin et al., 1987; Fenart et al., 2006; Ferrant & Bouharmont, 1994; Hallden et al., 1991; Hornsey, 1973a; Ivanov et al., 2004; Ivanov et al., 2005; Khvorostov et al., 2001; Kubo et al., 1999; Laporte et al., 2001; Lorenz et al., 1994, 1997; Majewska-Sawka et al., 1993;

Owen, 1945; Ran & Michaelis, 1995; Sadoch et al., 2003; Saeglitz et al., 2000; Satoh et al., 2006; Saumitoulaprade et al., 1993; Smith & Ruppel, 1980; Weihe et al., 1991)

4.5.2. Apomixis

(Fang et al., 2004) constructed a plant-transformation-competent binary BAC library for the B. corolliflora chromosome 9 monosomic addition line in sugar beet (M14). This library was estimated to have an average insert size of 127 kb and to be equivalent to 7.5 haploid genomes of the addition line, which contains not only the entire genome of sugar beet, but also the B. corolliflora chromosome 9 carrying the genes responsible for apomixis. Therefore, this library will be useful for isolation of the genes for apomixis in M14, as well as for genome research in sugar beet in general. Furthermore, because the library was cloned into an Agrobacterium-mediated, plant-transformation-competent binary vector pCLD04541 (Jones et al., 1992; Tao & Zhang, 1998), it can be directly transformed into plants via Agrobacterium. Therefore, this library will streamline the identification of apomixis genes and large-scale functional analysis of sugar beet genome sequences by transformation. The authors identified the BACs that originated from the B.corolliflora chromosome 9 and developed a sublibrary (bcBAC-IX) of clones specific for this chromosome. The sublibrary contains a total of 2,365 clones, providing genome coverage of approximately 3.8 equivalents of the alien chromosome (80 Mb). The genome coverage of the sublibrary is highly consistent with that which was expected. This sublibrary represents an important resource for the molecular characterization of the alien chromosome and the final isolation of the genes for apomixis. However, it is possible that this sublibrary may not completely cover the whole alien chromosome.

(Fang et al., 2004) give furthermore some final remarks about results: Successful transformation of large DNA fragment via binary BAC or TAC has been reported by (Hamilton et al., 1996; Hamilton et al., 1999; Liu et al., 1999). This system would make it feasible to study the expression of plant genes or gene clusters in their native genomic context and might eliminate genomic site-dependent gene expression. Therefore, it would be applicable for the isolation of genes that encode complex quantitative traits as well as genes within complex loci located in a chromosomal region of low recombination frequency (Hamilton et al., 1996; Liu et al., 1999). Combined with the physical map and genetic delimitation of the chromosomal region in which the apomixis locus resides, the genes for apomixis could be identified by genetic transformation of successive binary BAC clones in the contigs, or selective transformation of candidate binary BAC clones containing ESTs of interest within the delimitated chromosomal region. Thus, the practically impossible long distance chromosome walking and precisely positioning of the apomixis gene(s) in the conventional positional cloning could be avoided. Further literature on apomixis: (Bruun et al., 1995; Gao & Jung, 2002; Jassem, 1976; Jassem & Jassem, 1971; ReamonButtner et al., 1996)

4.5.3. Monitoring schemes

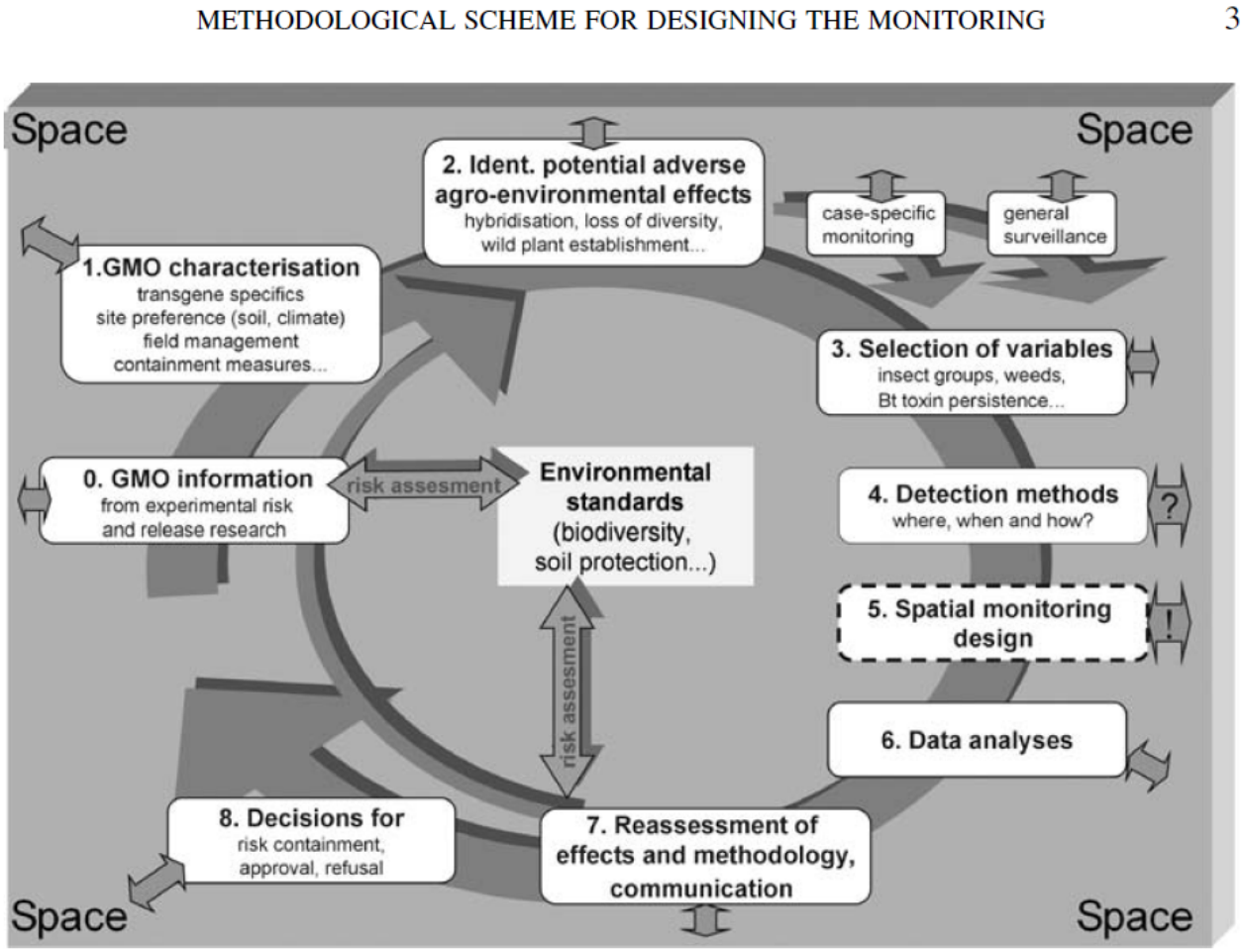


Fig. 23 StepwiseGMOmonitoring and assessment approach (Umweltbundesamt, 2001, modified), from (Graef et al., 2005).

(Graef et al., 2005) propose a monitoring system as shown in fig. 18 with the following steps: (1) characterisation of the market-ready GM crop from release-related risk research information; (2) information on potential adverse environmental effects. Both (1) and (2) are part of the environmental risk assessments prior to environmental release; (3) selection of indicators for anticipated adverse effects, in the following referred to as variables. The choice of variables to be monitored should be scientifically based, in particular depending on their exposure and on their indicator value. Various research groups, e.g. (Firbank et al., 2003; Romeis et al., 2006; Saeglitz et al., 2006; Zueghart & Breckling, 2003) have proposed concepts for selecting monitoring variables for potential agro-environmental effects of GMplants; (4) analytical methods by which these can be measured and evaluated; (5) adapted spatial design of a GMcrop monitoring network. Once GM crop monitoring is carried out, (6) measurement data are produced and analysed. From the results (7) subsequent risk (re)assessments are carried out to derive (8) decisions on risk containment, approval or refusal of the GM plant and to possibly adapt the monitoring methodology.

BETA GENE FLOW ASSESSMENT

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) southern Europe yes, northern Europe no	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
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
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	**Propensity for successful seed- and tiller mediated gene flow from commercial crop to feral**	
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)**	**3 (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,**

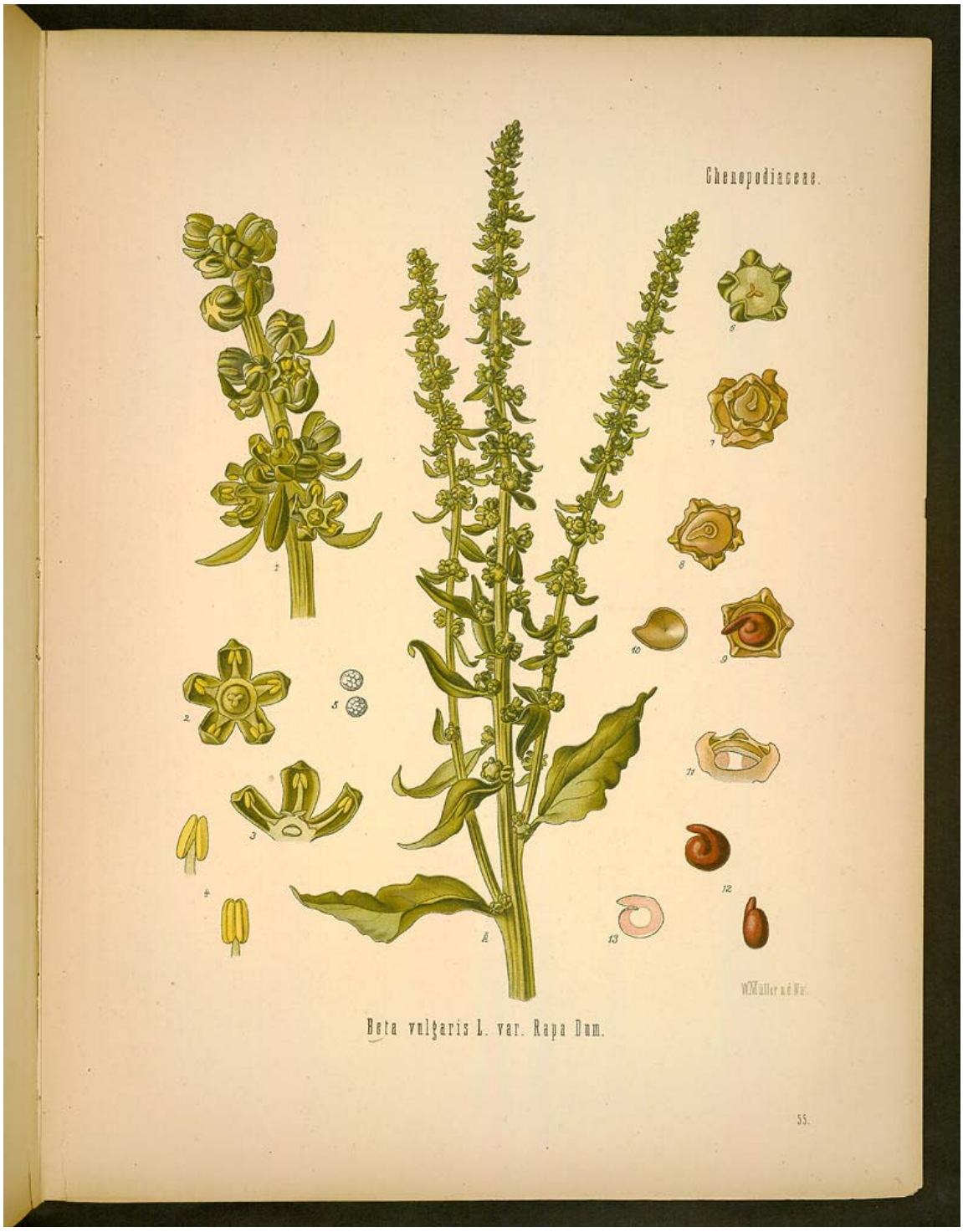


Fig. 24 Beta vulgaris, var. Rapa Dum. 55 common beet Missouri Botanical Gardens, Rare Books: From Köhlers Medizinalpflanzen in naturgetreuen Abbildungen mit kurz erläuternden Texten, 1883-1914
http://www.illustratedgarden.org/mobot/rarebooks/page.asp?relation=QK99A1K6318831914B1&identifier=0344

Brassica, Oilseed Rape

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.

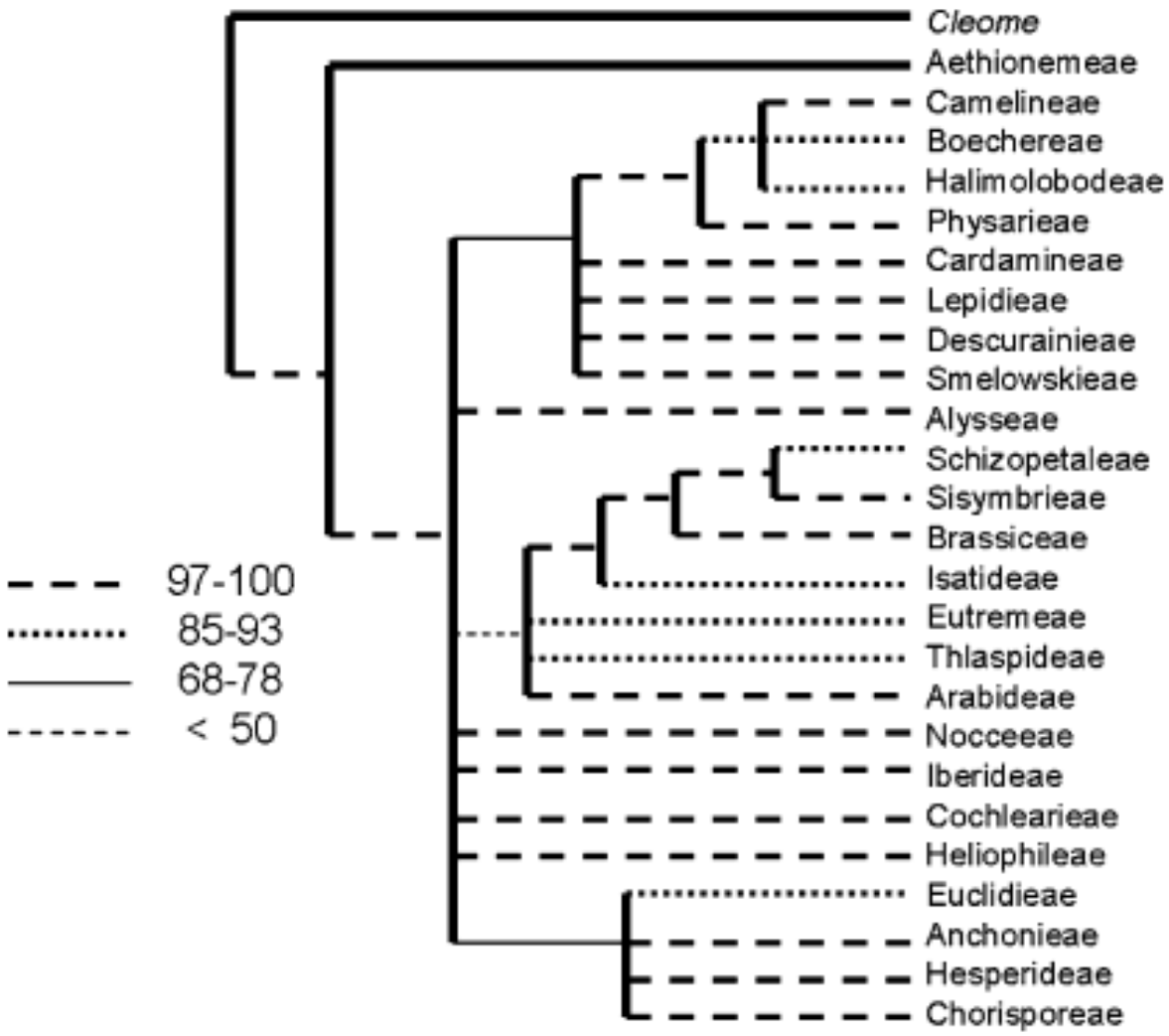


Fig. 25 Phylogenetic relationships among tribes of the Brassicaceae (modified from (Beilstein et al., 2006).

1.2.2. Phylogenetic relationships within Brassicaceaes

(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 erucoides, 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. erucoides, and S. aucheri - were closely related, advanced in the lineage, and were the closest apparent relatives (particularly D. erucoides) 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.

According to (Cheung et al., 1997) the genus *Brassica* consists of several hundreds of diploid and amphidiploid species. Most of the diploid species have eight, nine or ten pairs of chromosomes, known respectively as the B, C, and A genomes.Genetic maps were constructed for both *B*. *napus* and *B*. *oleracea* using mostly RFLP and RAPD markers.For the *B*. *napus* linkage map, 274 RFLPs, 66 RAPDs, and two STS loci were arranged in 19 major linkage groups and ten smaller unassigned segments, covering a genetic distance of 2125 cM. A genetic map of *B*. *oleracea* was constructed using the same set of RFLP probes and RAPD primers. The *B*. *oleracea* map consisted of 270 RFLPs, 31 RAPDs, one STS, three SCARs, one phenotypic and four isozyme marker loci, arranged into nine major linkage groups and four smaller unassigned segments, covering a genetic distance of 1606 cM. Comparison of the *B*. *napus* and *B*. *oleracea* linkage maps showed that eight out of nine *B*. *oleracea* linkage groups were conserved in the *B*. *napus* map. There were also regions in the *B*. *oleracea* map showing homoeologies with more than one linkage group in the *B*. *napus* map. These results provided molecular evidence for *B*. *oleracea*, or a closely related 2n”18 *Brassica* species, as the C-genome progenitor, and also reßected on the homoeology between the A and C genomes in *B*. *napus*.

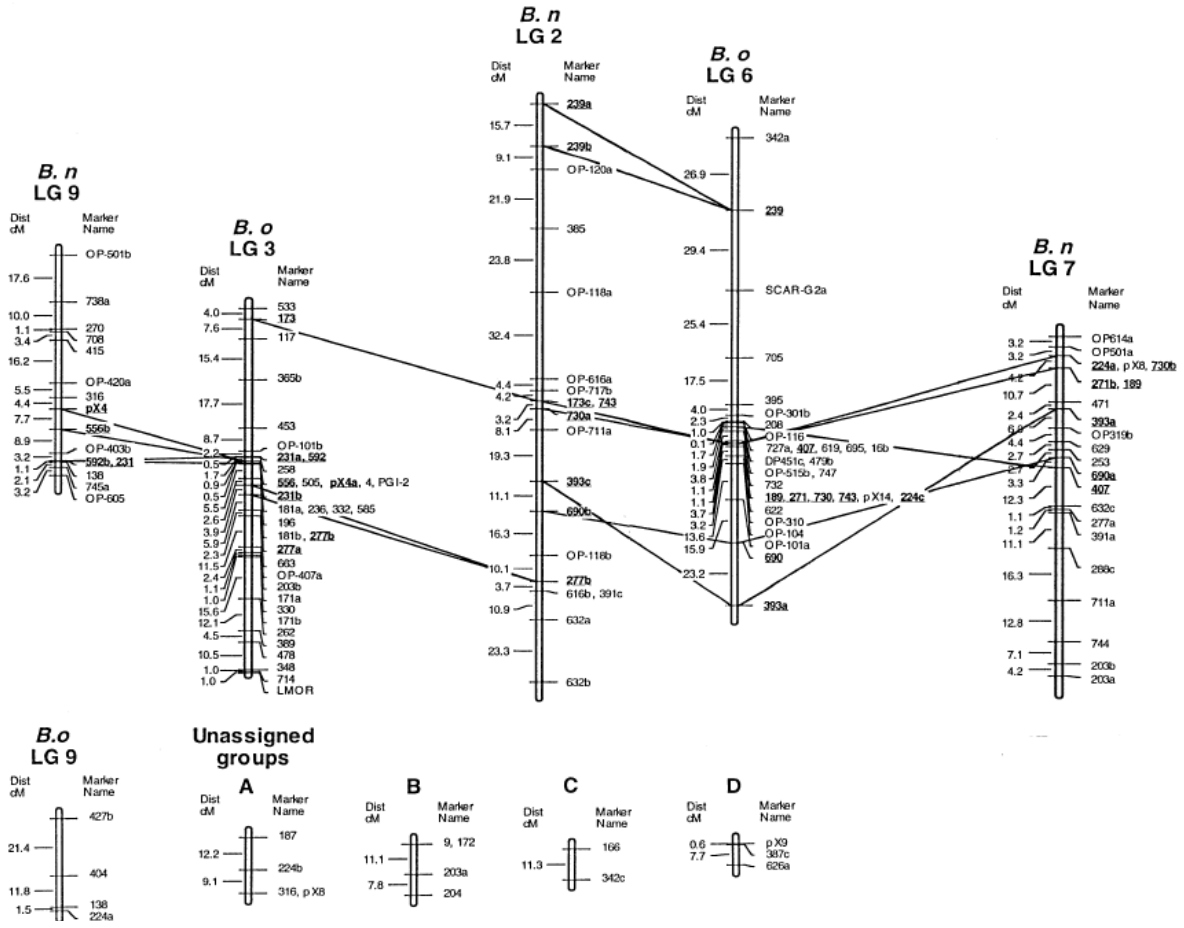


Fig. 26 Comparison of the genetic maps of *B. napus* and *B. oleracea*. Major conserved regions between the two genomes are shown with reference to the *B. oleracea* map. Common markers are *underlined* and joined by *solid lines* between the corresponding *B. oleracea* (*B.o*) and the *B. napus* (*B.n*) linkage groups. From (Cheung et al., 1997)

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. his correlation is significant because of the potential predictive value orcpDNA data in delimiting cytodelnes 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.

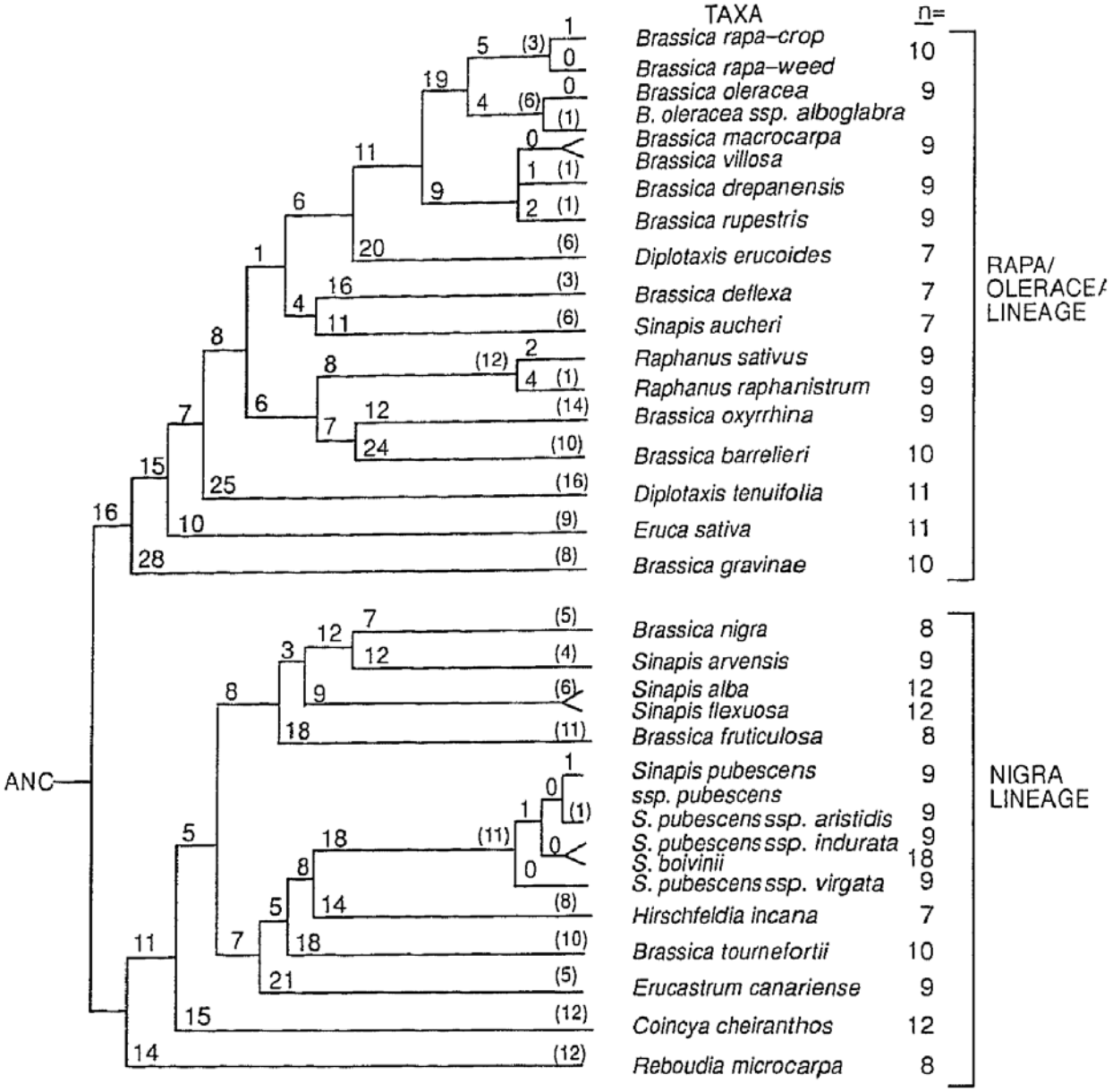
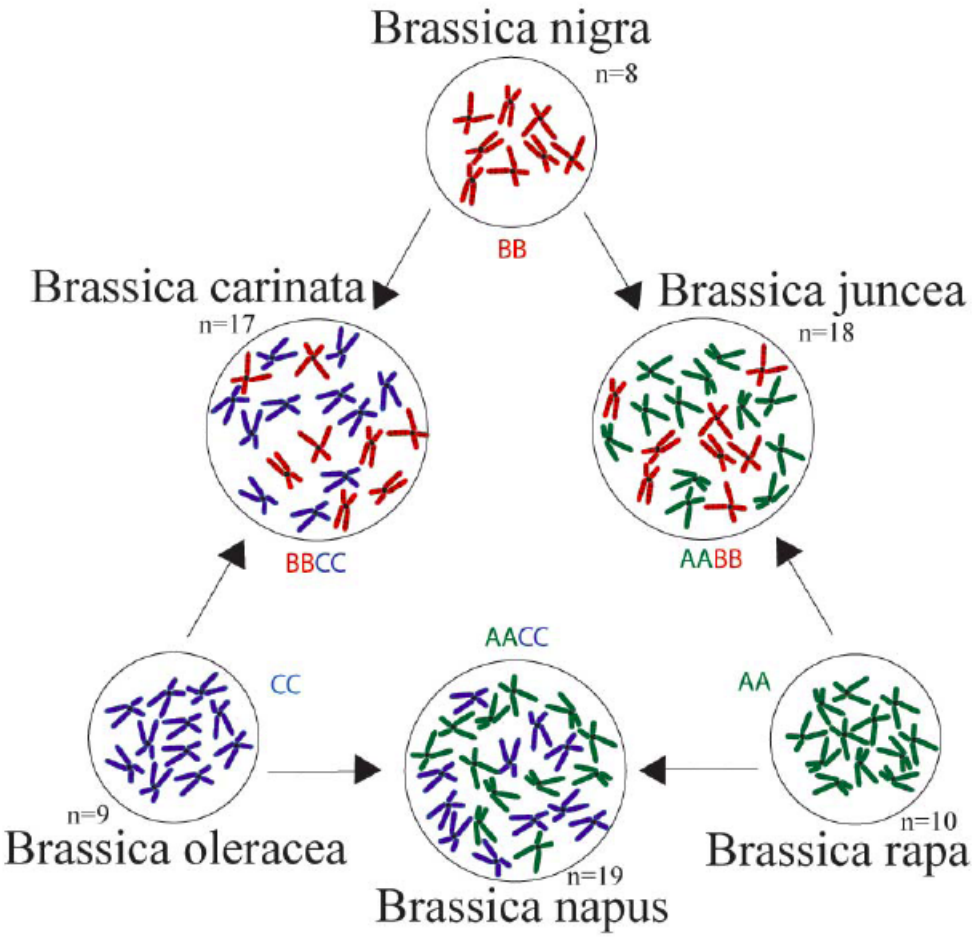


Fig. 27 Selected phylogenetic tree for the Subtribe Brassiciilae based on PAUP analyses of the chloroplast DNA restriction site/length mutations in the Appendix, which are shared by two or

more laxa/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 evolutution 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 lengt. ANC shows the common hypothetical common ancestor. From (Warwick & Black, 1991).

1.2.4. Relationships within the genus Brassica



The Triangle of U, where U is the transliterated author name, (U, 1935) is a theory about the evolution and relationships between members of the plant genus *Brassica*. It says that the genomes of three ancestral species of *Brassica* combined to create the three common vegetables and oilseed crop species that we know today. The theory has since been confirmed by studies of DNA and proteins.

The theory was first published in 1935 by Woo Jang-choon, a Korean botanist who was working in Japan (where his name was transliterated as “Nagaharu U” (U, 1935). Woo made synthetic hybrids between the diploid and tetraploid species and examined how the chromosomes paired in the resulting triploids. His work was influenced by work by (Kihara, 1962, 1965) on the origin of bread or hexaploid wheat and its relationship to its diploid ancestors.

The triangle shows how three of the *Brassica* species were derived from three ancestral genomes, denoted by the letters AA, BB, or CC. Alone, each of these diploid genomes produces a common *Brassica* species. The letter *n* denotes the number of chromosomes in each genome, and is the number found in the pollen or ovule. For example *Brassica rapa* has an A - n=10 (alternatively AA - 2n=20) designation. That means each somatic cell of the plant contains two complete genome copies (diploid) and each genome has ten chromosomes. Thus each cell will contain 20 chromosomes; since this is the diploid number it is written as 2n = 2x = 20.

AA - 2n=2x=20 - Brassica rapa (Brassica campestris) - Turnip, Chinese cabbage
BB - 2n=2x=16 - Brassica nigra - Black mustard
CC - 2n=2x=18 - Brassica oleracea - Cabbage, kale, broccoli, cauliflower
These three species exist as separate species. But because they are closely related it was possible for them to interbreed. This interspecific breeding allowed the creation of three new species of tetraploid Brassica. Because they are derived from the genomes of two different species, these hybrid plants are said to be allotetraploid (contain four genomes, derived from two different ancestral species). (Data from molecular studies indicate that the three diploid species are themselves paleopolyploids).

- AABB - 2n=4x=36 -Brassica juncea - Indian mustard
- AACC - 2n=4x=38 -Brassica napus - Rapeseed, rutabaga
- BBCC - 2n=4x=34 -Brassica carinata - Ethiopian mustard
-

Geographic distribution and Hypothetical origin, evolutionary pathways of B.oleracea and B. rapa after (Song et al., 1990)

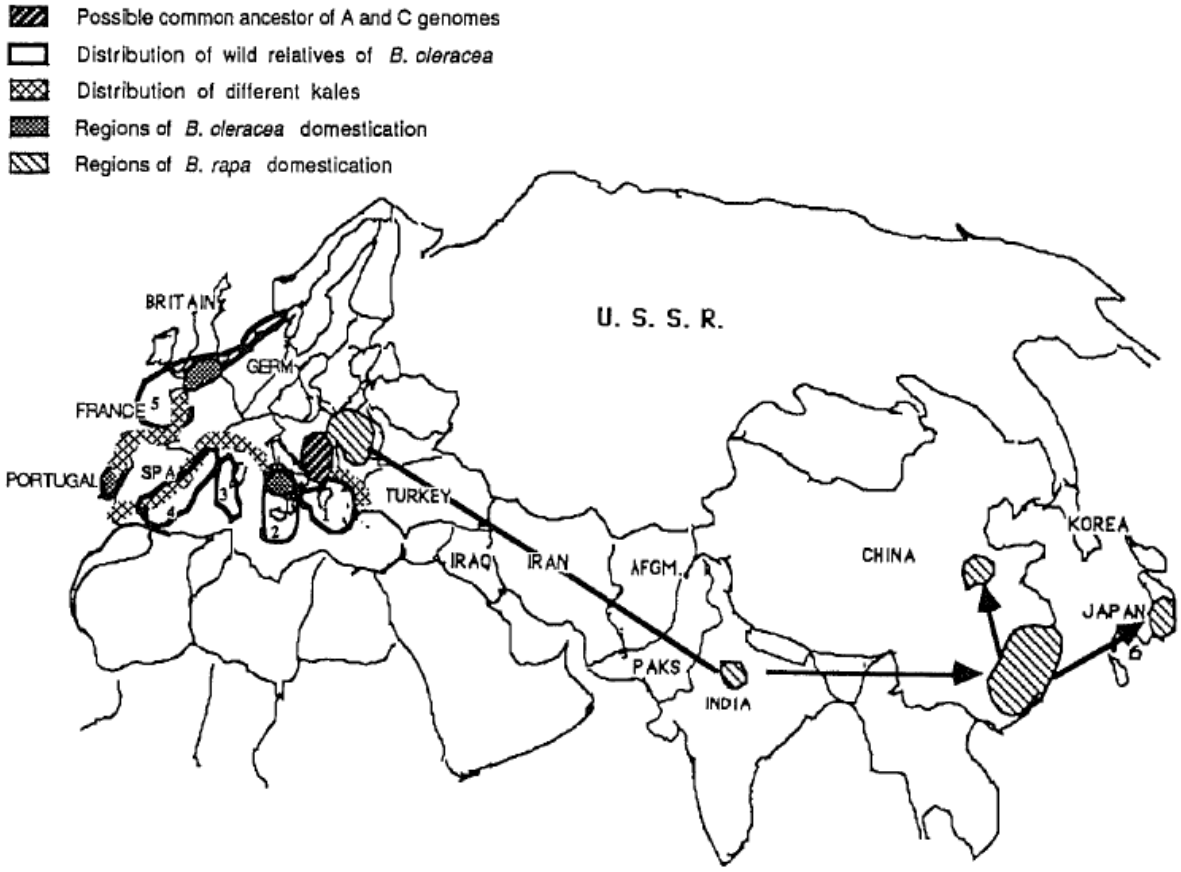


Fig. 28 Geographic distribution, and hypothetical origin and evolutionary *pathways of B. oleracea and B. rapa.* B. oleracea and B. rapa might have derived from a common ancestral species in Europe. B. *rapa* was then disseminated southeast and formed different centers of diversity, whereas B. *oleracea* spread out along the Mediterranean coasts to England and France. The numbers in white boxes indicate natural distribution of wild n = *9* brassicas used in this study (according *to Snogerup 1980): 1. B. cretica;* 2. *B. rupestris-incana*-complex; 3. *B. insularis*; 4. *B. montana*; and 5. *B. oleracea* from (Song et al., 1990)

An interesting hypothetical scheme of genome relations after (Song et al., 1990)

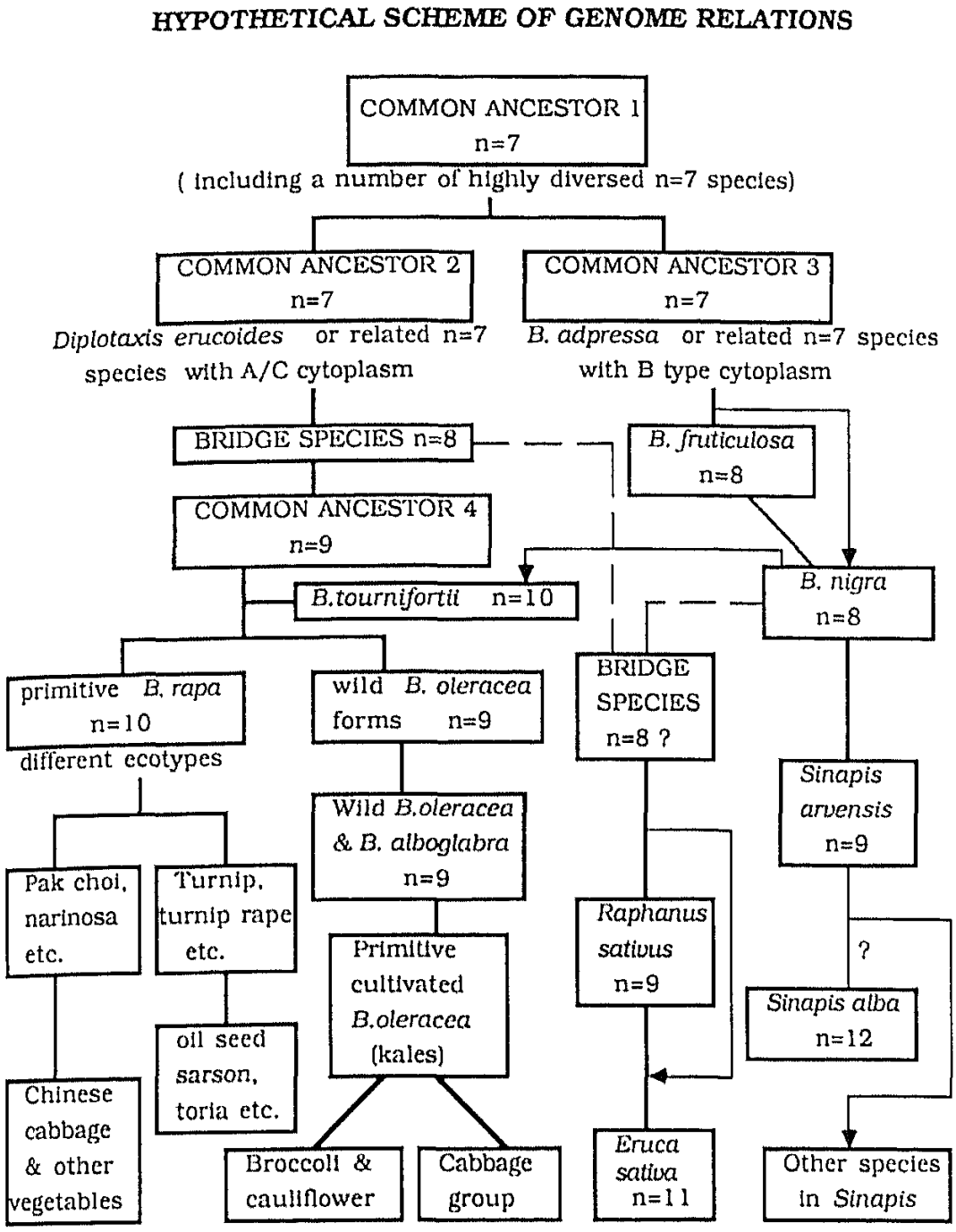


Fig. 29 Hypothetical scheme of genomic relations of *Brassica* and related genera based mainly on analysis of accessions used in this study. The *solid thick lines* indicate the main directions of genome evolution. The *solid thin lines* with *arrows* indicate the possible alternative pathways or introgression. *Dashed lines* indicate possible hybridization (see text for details). A/C and B cytoplasms were determined by the same criteria as in our previous report (Song et al. 1988 a) from (Song et al., 1990)

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 developped, 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., 2005b) 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.

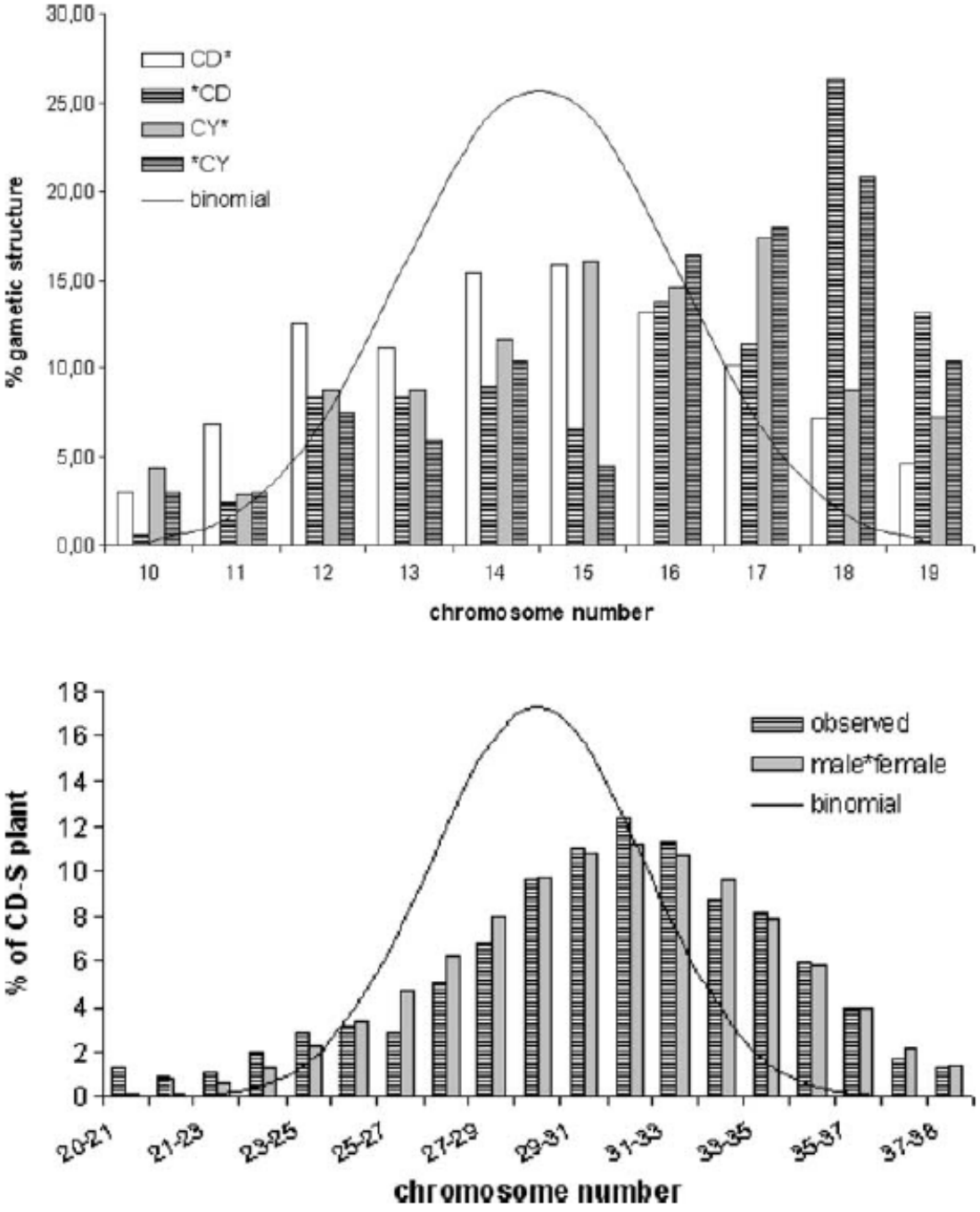


Fig. 30 above: Frequency of gametes with each chromosome number where triploid hybrids are male or female. Unreduced gametes were excluded from this analysis. Below: Distribution of chromosome number in CD-S plants

Chi-square analyses indicated that none of the observed distributions of gametic structure corresponded to expectations (a = 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 chromo somes 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 (a = 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 istribution 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 & Aguinagalde, 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, 1997b; Warwick et al., 2006; Zeng et al., 2004; Zhao et al., 2005b; 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

2.3. 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 F 1 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.

(Legere, 2005; Legere et al., 2006) show that control of herbicide tolerant canola is easy outside the application of Roundup Ready: Their studies of HR gene flow have provided background information that should be useful in the assessment of novel traits that may have more disruptive effects. In the absence of the herbicide, HR is quite a neutral trait when compared with salt or drought tolerance, insect or disease resistance.

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.

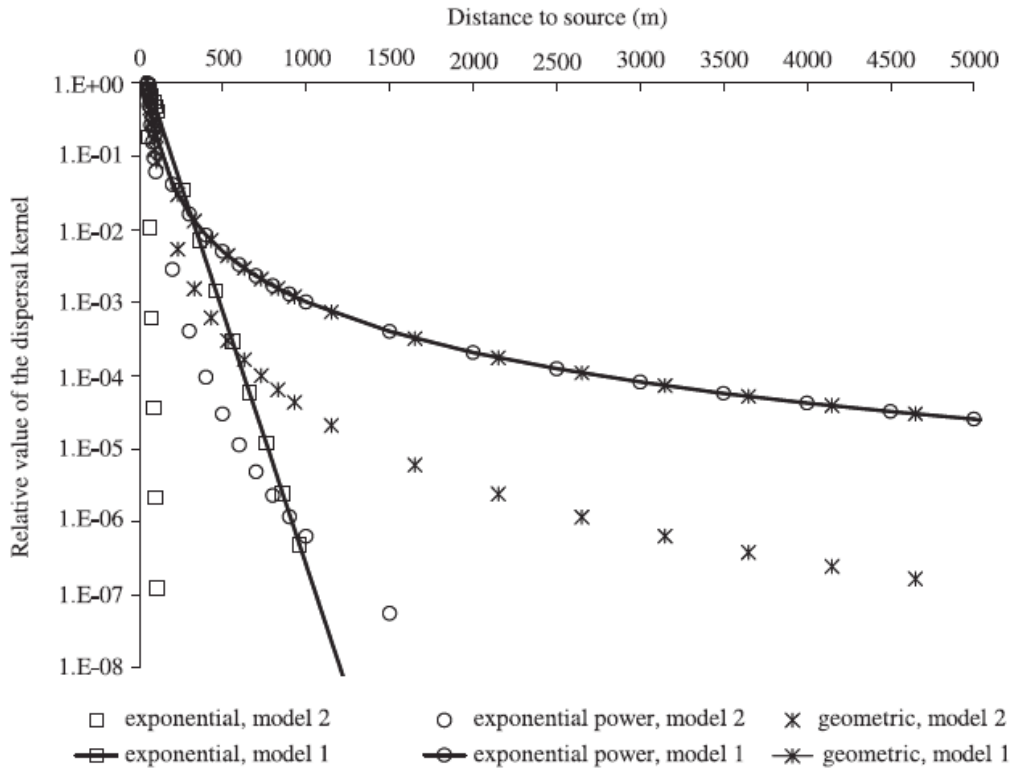


Fig. 31 Relative values of the dispersal kernels, against distance to source, estimated for models 1 and 2 on the experimental data. Open squares denote the exponential functions, open circles the exponential power functions, and crosses the geometric functions. All functions were normalized to take 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 km ^{2} 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.

2.4. 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.

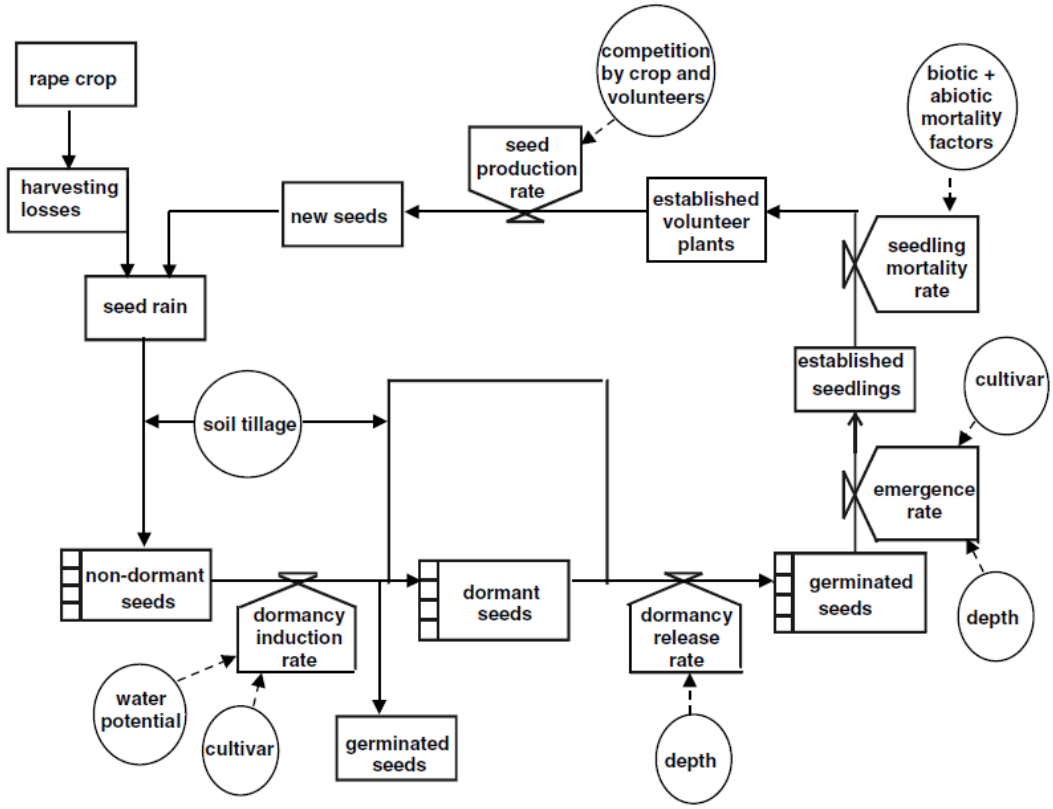


Fig. 32 General structure of the model. Boxes represent individuals (seeds or volunteer plants). Values represent rates that influence the transition from one step to the next. Within the circles factors are depicted that influence transition rates. From (Pekrun et al., 2006)

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	**Propensity for successful seed- and tiller mediated gene flow from commercial crop to feral**		
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**

Crambe, Seakale

1. Taxonomy

Crambe as a genus has been created by Linné Species Plantarum vol.2, 1753, the Type is not designated. (International Plant Names Index, 2004)

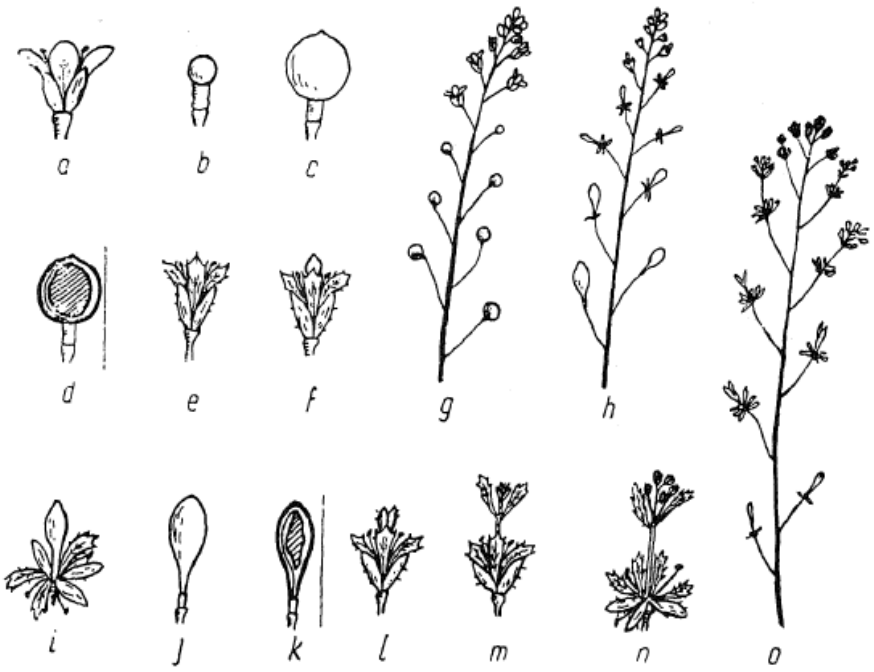


Figure 1. Schematic drawing showing the development of a normal green and proliferating flower of *Crambe abyssinica*.

a. Diagram of normal flower
b. The origins of a reduced capsule
c. Ripe capsule
d. Cross-section of ripe capsule
e. f. Green-pigmented flower (parts of flower assuming the properties and shape of leaves)
g. Apex of normal shoot
h. Apex of shoot with deformed capsules
i. Initial stage of deformed capsule with remains of green perianth
j. Ripe deformed capsule
k. Ripe deformed capsule (cross-section)
l. Initial stage of separation of carpels
m. n. Proliferation of initial stage; one (m) or several (n) flowers which become green are growing between the parted carpels.
o. Apex of shoot with separating carpels.

(By kind permission of Dr. T. Glover, Warsaw)

Fig. 33 Schematic drawing showing the development of a normal green and proliferating flower of Crambe abyssinica, by Dr T. Glover, Warsaw. From (Cornelius & Simmons, 1969)

(Francisco-Ortega et al., 1999) note: *Crambe* L. (Brassicaceae) is an OldWorld genus with a disjunct distribution among four major centers of species diversity.Aphylogenetic analysis of nucleotide sequences of the internal transcribed spacers (ITS) of the nuclear ribosomal repeat was conducted with 27 species of *Crambe* and 18 related genera. Cladistic analyses using weighted and unweighted parsimony support *Crambe* as a monophyletic genus with three major lineages. The first comprises those taxa endemic to the Macaronesian archipelagos. Taxa with a predominantMediterranean distribution form the second assemblage, and a disjunction between east Africa (*C. abyssinica*) and the Mediterranean (*C. hispanica*) occurs in this clade. The third lineage includes all Eurosiberian–Asian taxa and *C. kilimandscharica,* a species from the highlands of east Africa. A basal biogeographic split between east Africa and Eurasia is present in the third clade. The patterns of relationships in the ITS tree are concordant with known climatic events in northern Africa and southwestern Asia since the middle Miocene. The ITS trees are congruent with the current sectional classification except for a few members of sections *Crambe, Leptocrambe,* and *Orientecrambe* (*C. cordifolia, C. endentula, C. kilimandscharica,* and *C. kotschyana*). Low levels of support in the basal branches do not allow resolution of which genera of the subtribes Raphaniae or Brassicinae are sister to *Crambe.* Both subtribes appear to be highly polyphyletic in the ITS trees.

1.1. Chromosome numbers

Chromosome numbers have been published by (White & Solt, 1978): Below the table of the original publication:

Table 1. Chromosome numbers and sources of *Crambe hispanica, C. kralikii, Crambella teretifolia,* and *Hemicrambe fruticulosa* accessions.

Species	P.I. no.	Original source	Chromosome no. (n)
C. hispanica var. *hispanica*	388708	Israel	30
	388710	Israel	30
	388720	Israel	30
	388724	Israel	30
	388728	Israel	30
	388822	Cyprus	30
	388832	Italy (Gargano)	30
	388835	Italy (Sardinia)	30
C. hispanica var. *glabrata*	388784	Morocco	15
	388785	Morocco	15
	388850	Portugal	15
	388855	Portugal	15
	388871	Spain	15
	388874	Spain	15
	392073	Spain	15
C. kralikii	372930†	Morocco	30
	378145†	Algeria	45
	388795	Morocco	30
	388796	Morocco	30
	388797	Morocco	15
	388798	Morocco	30
	388799	Morocco	30
	388800	Morocco	30
	388801	Morocco	30
	388802	Morocco	30
Crambella teretifolia	†	Morocco	11
Hemicrambe fruticulosa	†	Morocco	9

† Seed provided by Dr. Cesar Gomez-Campo, University of Madrid.

Fig. 34 Chromosome numbers and sources of Crambe hispanica, C. kralikii, Crambella teretifolia and Hemicrambe fruticulosa accessions. From (White & Solt, 1978).

1.2. Monophyly and Origin of Crambe

Brassica, Crambe, Diplotaxis, and Erucastrum are the only genera of the Brassiceae with more than 10 species. Among these genera, *Crambe* is the only genus that has been shown to be monophyletic using molecular data (Warwick & Black, 1991, 1997b; Warwick et al., 1992). Chloroplast DNA restriction site data provided only weak support (30% bootstrap value) for the monophyly of *Crambe* (Warwick & Black, 1997b). Our analysis of ITS variation provides stronger support for the monophyly of the genus (bootstrap values ranged between 63 and 71%).

Both cpDNA restriction site and morphological data suggest that *Crambe* may represent a distinct subtribe within the Brassicaceae (Gomez-Campo, 1980) (Warwick & Black, 1997b). The ITS data do not resolve this issue because relationships among genera of the Brassiceae are weakly supported in most cases. No single genus is sister to *Crambe* in the ITS trees. Previous suggestions of a close phylogenetic relationship between *Crambe* and *Calepina* (Clemente & Hernandez-Bermejo, 1978a, b, 1980a, b)

or *Hemicrambe* (Gomez-Campo & Tortosa, 1974) are not supported by the ITS data. Our phylogeny agrees with other hypotheses (Gomez-Campo, 1980) (Warwick & Black, 1997a), which suggested that *Calepina* should not be considered part of the Brassiceae.

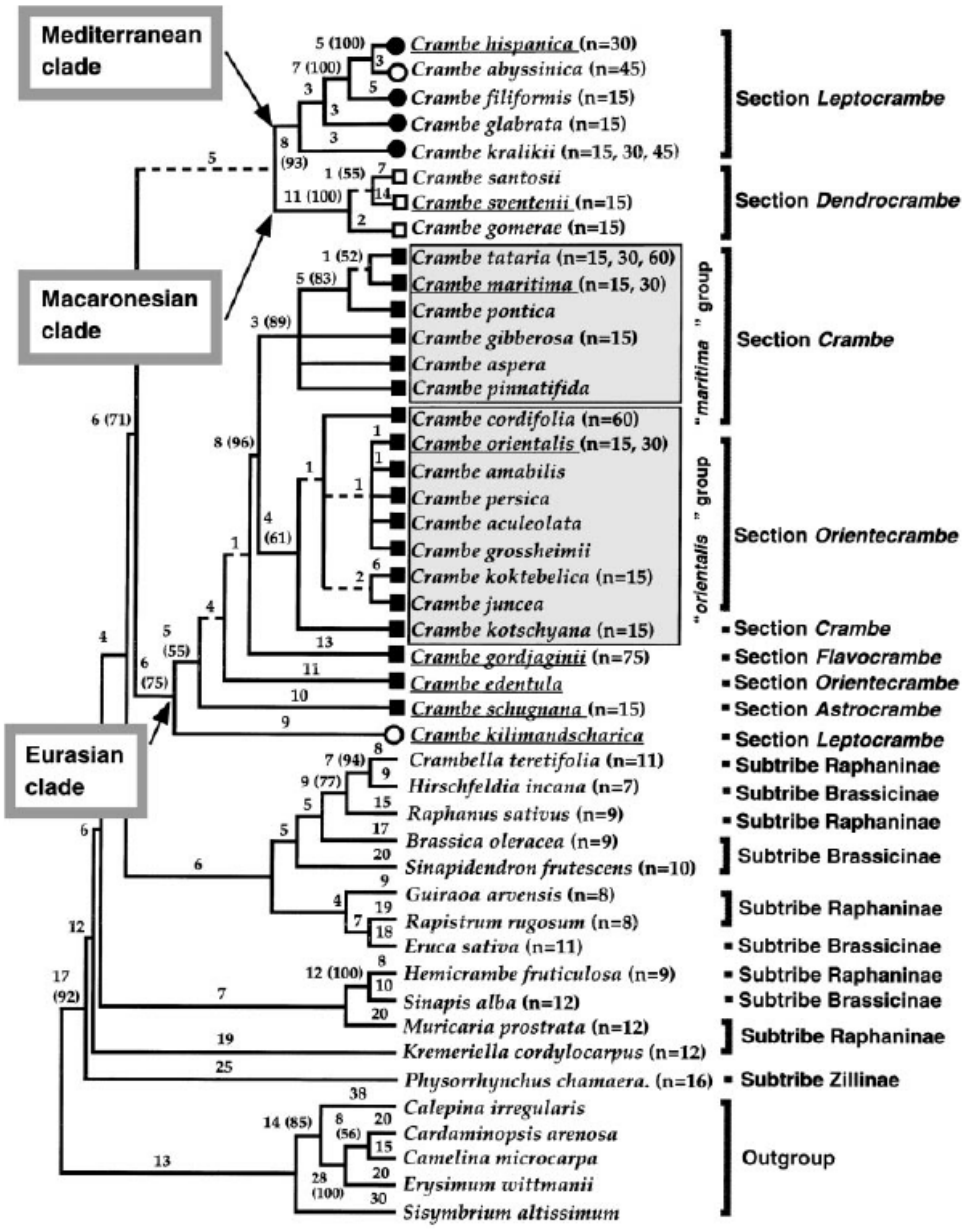


Fig. 35 One of the 1491 shortest trees from the unweighted parsimony analysis that treated indels as missing data (637 steps; CI 5 0.478, without autapomorphies; RI 5 0.681). Branches which collapse in the strict consensus tree are indicated by dashed lines. Number of changes are indicated along each branch. Bootstrap values higher than 50% are indicated in parenthesis. Distribution of *Crambe* species are: closed circles, Mediterranean; open circles, east African; open squares, Macaronesian; closed squares, Eurasian. Haploid chromosome numbers are indicated in parentheses when known. Species examined to test the molecular clock hypothesis are underlined. From (Francisco-Ortega et al., 1999).

1.3. Sectional Classification

The main incongruence between the ITS phylogeny and the current sectional classification concerns *C. kilimandscharica.* This annual species from east Africa has been traditionally considered part of sect. *Leptocrambe.* In addition, this species has small, rugose fruits that are virtually identical to most of the Macaronesian species. *C. kilimandscharica,* however, is the basal member of a clade which includes the four Eurosiberian–Asian sections of Khalilov (1991a,b). This placement may indicate that the morphological similarities between *C. kilimandscharica* and sect. *Leptocrambe* may be homologous and plesiomorphic for the Eurasian clade.

Another disagreement between the ITS trees and the current sectional classification concerns the monophyly of the four Eurosiberian–Asian sections (Khalilov 1991a,b). These sections are clearly not monophyletic in the ITS tree (Figs. 1–3). However, the ITS phylogeny is congruent with the earlier classification of Schulz (1919), which recognized one section for all taxa of the Eurasian clade with the exception of *C. kilimandscharica.* Within the Eurasian clade, the ITS trees identify two major lineages, here designated ‘‘*maritima*’’ and ‘‘*orientalis*’’. These two groups are also geographically distinct. All species in the ‘‘*maritima*’’ group have European distributions and do not occur east of the Caucasus region. In contrast, species in the ‘‘*orientalis*’’ group have a predominant Asian distribution. The major disagreement between all previous treatments of the Eurasian taxa and the ITS trees concerns *C. edentula.* This species has been suggested to be closely related to *C. orientalis* by (Khalilov, 1991a, b). However, the ITS phylogenies do not place it in the ‘‘*orientalis*’’ group.

The ITS trees and previous taxonomic treatments (Candolle, 1821, 1824) (Schulz, 1936) (Gomez-Campo, 1980) (Khalilov, 1991a, b) concur that the Macaronesian taxa are monophyletic. They also indicate a close phylogenetic relationship among the Mediterranean species (including *C. abyssinica*). The sister relationship of *C. hispanica* and *C. abyssinica* has strong support, confirming previous hypotheses of the taxonomic proximity of these species (White, 1975) (Jonsell, 1982). See also (Inaba & Nishio, 2002)

(Prina, 2000) published a recent taxonomic revision of the genus Crambe, sect. Leptocrambe, Brassicaceae:
As part of a revision of the genus Crambe based on the morphological study of herbaria and cultivated material, the systematics of sect. Leptocrambe DC. is presented. Section Leptocrambe is considered to comprise five species: C. kilimandscharica O. E. Schulz, C. sinuatodentata Hochst. ex Petri, C. hispanica L., C. filiformis Jacq. and C. kralikii Coss. C. hispanica includes three subspecies, subsp. hispanica, subsp. glabrata (DC.) Cout. and subsp. abyssinica (Hochst. ex R. E. Fr.) stat, nov. which includes var. abyssinica and var, meyeri (O. E. Schulz) comb. nov. C. kralikii includes two subspecies, subsp. kralikii and subs;. garamas (Maire) Podlech.

1.4. Case study on Crambe abyssinica

The example of Crambe abyssinica (Mediterranean clade, see fig. 3): (Cornelius & Simmons, 1969):

The first investigations into the possible uses of *Crambe abyssinica* as an oilseed were in Russia in 1932, in a programme of research for new sources of oil for domestic and industrial uses and a comprehensive study and trials have since been carried out in that ~ountry.~TJr~ia ls have also been undertaken in the United States since the early part of 1960194 and several other countries including Canada, Venezuela, Poland, Sweden, Kenya and Northern Nigeria have also experimented with cultivation.

Production in the U.S.A. is now on a commercial basis due to chemical removal of unwelcome toxic substances in the processed seeds: (Mustakas et al., 1968a; Mustakas et al., 1968b; Mustakas et al., 1976; Mustakas et al., 1964, 1965).

Botany of Crambe abyssinica:

In the wild form, *Crambe* is distributed in the Abyssinian foothills and the North African plains. It grows as a weed in cultivated fields, under trees, and in the bush along the borders of field paths, generally singly and not forming a continuous growth.
The genus *Crambe* belongs to the Cruciferae family; the genus includes twenty-nine species, but only C. *abyssinica* is cultivated. It is an annual herbaceous plant, branching abundantly in natural conditions and in thin sowings. The average height in field conditions with sufficient rainfall is about one metre but somewhat shorter in dry situations. The lower part of the stem is covered with short, fine hairs but the upper part is glabrous. The leaves are lyrate, pinnately divided, with large terminal teeth of ovate shape. The fruit is of a pale yellow colour, containing a single spherical seed, greenish-brown or brown in colour and about 0.8 to 2.55 m in diameter. In ordinary field conditions one *Crambe* plant can produce 530 to 1,840 fruits.

For the following paragraphs on cultivation and production see (Cornelius & Simmons, 1969) and also (Lessman & Meier, 1972).

(Wang et al., 2000): *Crambe abyssinica* is an annual herb. After introduction and cultivation in China, the oil content of its whole seed reaches 34.48%, of which 62.50% is erucic acid. Some available characters, such as short growing season, potentiality of high yield and good resistance to diseases were found. In the Chengdu area, the experiment of sowing date and density indicated that the sowing date of *C*. *abyssinica* could be preliminarily determined: from the beginning of October to mid-October and 150 000 plants:ha was more suitable. After single-plant selection, two new strains named N01 and B07, respectively, were obtained. Multi-locational evaluation showed that N01 and B07 were superior to the original both in yield and stress resistance. Therefore, *C*. *abyssinica* shows itself a promising oil crop, which can be used in industry in China but further studies are needed.

(Fontana et al., 1998): The use of oil with a high level of erucic acid in some industrial sectors appears to offer excellent prospects from a technological and environmental point of view. This study was carried out to determine the potential of *Crambe abyssinica* for producing high erucic acid oil seeds in the Po Valley environment (North Italy). The

productive yield of 3-year trials using six different genotypes was generally satisfactory, even though significant differences were found between the years. The different weather patterns recorded during the trials showed how emergence, flowering and seed-filling stages are particularly important phases for obtaining good yields. The grain production in these years ranged between 2.3 and 3.2 t ha−1 and were similar to that of other spring oilseed crops in our environment. The seed had an oil concentration of between 320 and 370 g kg−1, with a fatty acid composition regularly characterized by a level of erucic acid higher than 53%. This paper also reports and discusses some other seed characteristics (protein content, weight per hectolitre, thousand kernel weight, hull/seed ratio and number of seed per plant). Amongst the genotypes tested, Mario gave the highest seed and oil yields followed by Belann and C-29, whereas Mejer and Belenzian gave lower yields, especially as a result of an insufficient emergence.

1.5. Molecular Taxonomy of Crambe

(Bond et al., 2005) used genetic analysis for the tracing of seed dispersal and population genetics: The main aims of this research were to investigate in C. maritima if population size influenced genetic diversity, if populations showed isolation by distance and if ocean currents could explain patterns of genetic differentiation. They have found no relationship between population size and genetic diversity and no relationship between geographic and genetic distance. However, patterns of genetic differentiation are related to ocean current in the region. In C. maritima, high levels of gene flow between populations in the English Channel and North Sea appear to maintain genetic variation. In contrast, populations on the Biscay coast have less opportunity for exchanging genes. The authors expect the patterns of population differentiation revealed here will be reflected in other coastal species whose seeds tolerate immersion in salt water for long periods of time. By using a genetic approach they have shown that it may not be accurate to view gene flow between coastal populations as strictly linear, especially when the seeds of the species examined tolerate immersion in seawater. Seed viability experiments do not prove that long distance seed dispersal actually happens; only that it is possible. Investigating population structuring using genetic techniques verifies if gene flow is, or has been, occurring between isolated populations. They have shown that detailed information about seed dispersal can be obtained using simple universal markers. In species with seeds dispersed by the sea, populations that are geographically close may be isolated because of currents, whilst geographically distant populations may be linked by currents. In light of our findings, it is important that the conservation of coastal species takes into consideration the possibility that gene flow may not occur between geographically adjacent populations. Our findings imply that gene flow between populations on either side of the English Channel can occur through the exchange of salt-water tolerant seeds.

(Briard et al., 2002): Seakale is a Brassicaceae, native to the coastal sands of Northwestern Europe. To bring this species closer to commercialisation and thereby enhance the diversification of vegetable crops, a breeding program was initiated in 1992. A systematic search for wild populations was undertaken in France, from Quiberon (south Brittany) to Dunkerque (north France near Belgium) to enlarge its genetic basis. Many sites previously described in the literature have disappeared, while five large sites,

not previously described, were found. Morphological descriptors and molecular markers (RAPD) were used to study the phenotypic and phenetic variability of the collected plants. A great variability for leaf and leaf-stalk colour, limb, flowers and siliques sizes, was observed. Among the wild collected plants, molecular similarity varied from 25 to 85%. The mean distance from all the wild genotypes to the breeding material already in collection was large (50%). Even if no clear correlation was found between morphological assessment and molecular data except for the leaf-stalk descriptor, the collecting trip was a success. A real enlargement of the variability was obtained.

2. Biosafety considerations

2.1. Gene flow in Crambe

There are only a few publications available on gene flow:

(Beck et al., 1975): A dominant marker gene controlling leaf pubescence has been used to measure outcrossing in crambe (Crambe abyssinica Hochst. Ex. R. E. Fries), a cruciferous crop species producing a seed oil with high content of erucic acid. In experimental plots, outcrossing rates within the range of 4.8 to 10.7 per cent have been determined in different types of plots between dominant and recessive genotypes. Overall outcrossing rates of 9 to 14.3 per cent considering both inter and intragenotypic cross-pollination, have been calculated.

(Beck et al., 1975) studied inheritance of pubescence and its use in outcrossing measurements between a Crambe-hispanica type and Crambe-abyssinica Hochst Ex Fries.

A rare experiment by (Vollmann & Ruckenbauer, 1991) revealed the following outcrossing rates in Crambe (Crambe-Abyssinica Hochst Ex Re Fries) Using a Dominant Morphological Marker Gene. In experimental plots, outcrossing rates within the range of 4.8 to 10.7 per cent have been determined in different types of plots between dominant and recessive genotypes. Overall outcrossing rates of 9 to 14.3 per cent considering both inter and intragenotypic cross-pollination, have been calculated.

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Table 2

Extent and estimations of outcrossing in crambe with respect to the type of plot and to replication

Plot	repl.	No. of plants glabrous	No. of plants total	% outcrosses	st. dev.	adjusted outcrossing rate
PL1	1	16	162	9.9	7.3	11.9
	2	25	216	11.6	7.2	13.9
PL2	1	9	186	4.8	6.4	14.5
	2	12	255	4.7	6.7	14.1
PL3	1	17	233	7.3	6.4	8.8
	2	19	245	7.8	8.0	9.3

Fig. 36 Extend and estimations of outcrossing in Crambe with respect to the type of plot and to replication, from (Vollmann & Ruckenbauer, 1991)

There are no further experiments published with the aim of determining gene flow for biosafety assessments. In this situation it will be advisable to rely on the vast experience with other Brassicaceae.

2.2. Some remarks about production, related to biodiversity and biosafety

(Wang et al., 2000): Increasing the yield of Crambe could be carried out by two ways, i.e. raising the planting density or the effective branch number. If the planting density was too high, the plant couldn't grow well and the primary branch number and the seed number per plant would decrease. Table 6 shows that the yield of 150 000 plants:ha was the highest, yield of 180 000 plants: ha was the second, and yield of 120 000 plants:ha was the lowest. Through LSR-method analysis the authors found that the difference between the yield of 150 000 plants:ha and that of 120 000 and 180 000 plants:ha was significant at 5% level, however, the difference between 120 000 and 180 000 plants:ha was not significant. Therefore, they suggested that the density of 150 000 plants:ha in the Chengdu area was more suitable.

(Wang et al., 2000):
Crambe oil is a good source of long-chain fatty acids and contains 10–15% more erucic acid than industrial rapeseed oil. Moreover, Crambe is a main self-pollination plant and it traverses cross pollination occurring between industrial rapeseed and canola or edible oil rapeseed in adjacent fields, which the result is an oil with intermediate erucic acid content of low value for industrial purposes and unfit for human consumption. Crambe introduced in China is an annual herb with higher seed yield and erucic acid content, and lower glucosinolate content. Therefore, Crambe shows promise of becoming a new oil crop with a high erucic acid content, which can be used in industry and an additional protein source as well in China. But problems with Crambe are weaker resistance to diseases and lack of required genetic diversity for improving it through breeding.

More recently, by the treatment of seed with different concentration and dose of EMS and ^{60}Co, the single-plant selection of M_1 generation was carried out.

(Kmec & Weiss, 1997):
A recent outbreak of the diamondback moth, Plutella xylostella (L.), in the northern Great Plains indicates this insect can be a serious pest of oilseed rape-Brassica napus L., Brassica rapa L.-and other cruciferous crops, including crambe, Crambe abyssinica Hochst, ex R. E. Fries. Despite the importance of this pest, there are no data on seasonal abundance of Pl. xylostella in the northern Great Plains. This information is essential for the design of adequate management strategies.

Responsive integrated pest management strategies require the establishment of an economic threshold. An effective sampling program is needed to determine whether the economic threshold has been reached. In the case of P. xylostella, an effective sampling technique is needed for larvae; however, direct counting of larvae is labor-intensive. Peak adult flight may be correlated with subsequent larval densities (Baker et al. 1982, Koshihara 1988). However, rainfall may k d many young larvae (Harcourt 1963, Wakisaka et al. 1991) and affects the relationship between adult and larval counts. **A** stronger relationship could be expected between the density of adults and eggs, because there is only a short time lag between the ***2*** life stages, and egg mortality is usually very low, ~0.9% (Harcourt 1986).

In the northern climates, cultivated host plants are not available to ovipositing P xylostella females early in the growing season, and the 1st generation develops on cruciferous weeds (data from Ontario; Harcourt 1957). The crop can be infested from the neighboring areas of cruciferous weeds or volunteers (Koshihara 1988, Rhan 1988). Therefore, a study of the seasonal dynamics of P. xylostella should start with the monitoring of cruciferous weeds.

The current study had 2 objectives-the first was to determine the seasonal abundance of Pl. xylostella during the growing season of crambe, and the second to identify an efficient method for estimating egg density.

Linum, Flax

1. Taxonomy

1.1. General taxonomy

(Muravenko et al., 2003) (reference No. see in link of citation) undertook genome comparisons with chromosomal and molecular markers for three closely related flax species and their hybrids.

The genus Linum L. comprises more than 200 species, including commercially valuable cultivated Linum usitatissimum L.
Taxonomy of the genus is complex and questionable [1–3]. Karyotypic analysis of Linum L. species started more than half century ago and allowed several flax species to be recognized [4–8]. It has been observed that chromosome number ranges from 12 to 72 in the genus, and that chromosomes are small (1–4 m m) and morphologically similar. Although monochrome staining reveals only general karyotypic differences, the results obtained with this method have made it possible to construct a putative phylogenetic tree both for the Old World and for New World species of the genus Linum [6, 8]. In addition, intra- and interspecific genetic diversity has been characterized with molecular (RAPD) [9–11] and protein [12, 13] markers, allowing certain taxonomic relationships to be assumed for several flax species. Notwithstanding, there are still numerous questionable issues in taxonomy and phylogeny of the genus Linum.
First and foremost, this is true for cultivated L. usitatissimum and its close or distant wild relatives, which are commonly assigned to the section Linum [2, 3, 14].

1.2. Taxonomy of Linum usitatissimum and related species

Taxonomy of cultivated L. usitatissimum and closely related species is rather complex and equivocal. It is believed that L. usitatissimum L. was domesticated approximately 6000 years ago [15, 16], and yet this species is still able to cross with its putative wild ancestor, Linum angustifolium Huds. [17, 18]. The two species each have 30 chromosomes in the karyotype. The same chromosome number is characteristic of Linum bienne Mill., which is also thought to have contributed to the origin of L. usitatissimum L. [16]. However, some authors consider L. bienne as a subspecies of L. angustifolium Huds., and some others even equate these two species [2, 3]. On the other hand, there is an opinion that L. bienne Mill. is a subspecies of L. usitatissimum L.

[19]. Hence, to eliminate this disagreement, it is necessary to employ modern molecular and cytogenetic techniques. Genetic polymorphism of the above three species has been assessed preliminarily by RAPD analysis [10]. Yet complex genome comparisons with the use of chromosome and molecular markers have not been carried out so far in order to clarify the taxonomic status and phylogenetic relationships of the three closely related flax species.

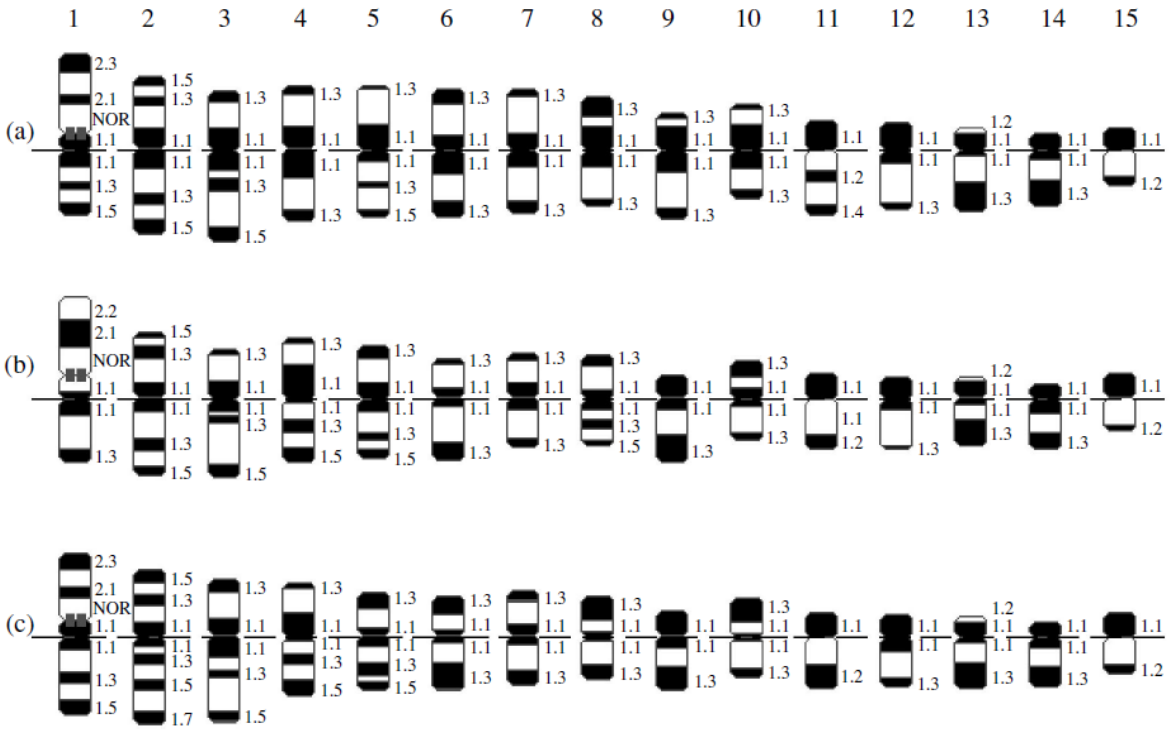

Fig. 37 Ideograms of C-banded chromosomes of (a) *L. angustifolium*, (b) *L. bienne*, and (c) *L. usitatissimum* cultivar Orshanskii 2. From (Muravenko et al., 2003)

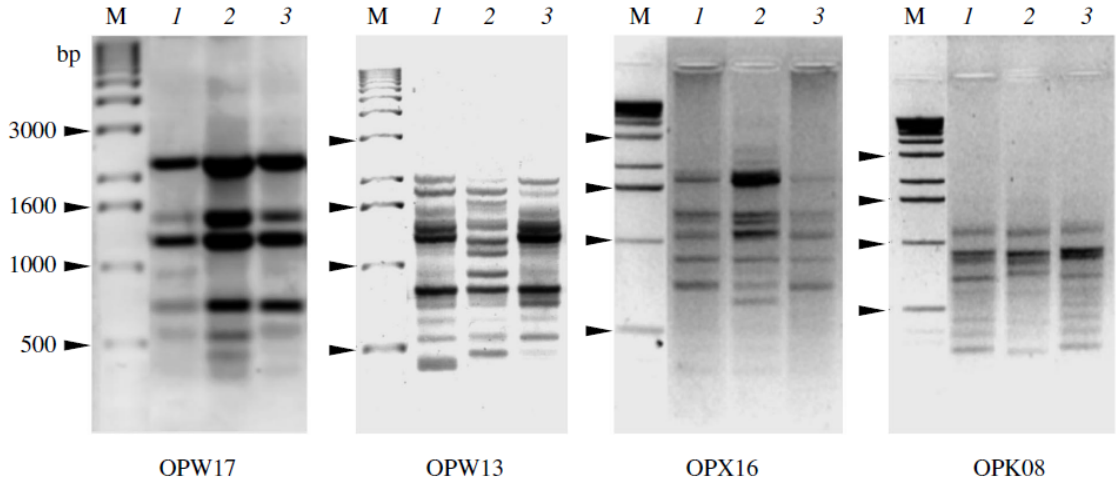

Fig. 38 RAPD patterns obtained with primers OPW17, OPW13, OPX16, and OPK08 for (*1*) *L. angustifolium*, (*2*) *L. bienne*, and (*3*) *L. usitatissimum.* M, molecular weight marker.

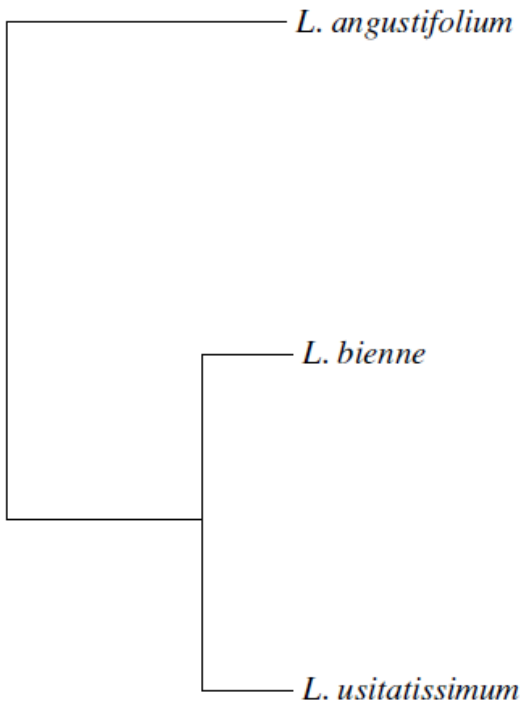

Fig. 39 Dendrogram of phylogenetic relationships of the three flax species, as inferred from analysis of genetic distances.

The RAPD patterns were used to construct a dendrogram of genetic similarity between the three flax species (Fig. 5). The genotypes under study formed two clusters, one including *L. usitatissimum* and *L. bienne* and the other combining these species with *L. angustifolium.* Genetic distance was 0.226 between *L. usitatissimum and L. bienne and 0.305 between L. angustifolium and L. usitatissimum and between L. angustifolium* and *L. bienne.* Thus, molecular analysis of genomic DNA showed that *L. bienne* clusters with *L. usitatissimum* and, therefore, cannot be considered as a separate species.

1.3. Taxonomy of Linum usitatissimum

(Fu et al., 2002):

Analysis of the extent and distribution of genetic diversity in crop plants is essential for optimizing sampling and breeding strategies.We used random amplified polymorphic DNA (RAPD) markers to assess genetic diversity and relationships in 22 Canadian cultivars, 29 selected world cultivars and 10 landraces of flax (*Linum usitatissimum* L.). RAPD variation was generally low and more variation was detected among, than within, the investigated flax accessions. Based on 53 variable RAPD loci observed for the 61 accessions, the landraces had a lower proportion of fixed recessive RAPD loci (0.427) (i.e., more genetic variation) than all of the flax cultivars examined (0.492). The linseed cultivars had a lower proportion of recessive loci (0.469) than the fiber flax cultivars

(0.529). Canadian linseed cultivars had a lower proportion of recessive loci (0.465) than the selected world flax cultivars (0.512). A trend was also observed that the rate of loss in genetic variation in Canadian flax breeding programs over the last fifty years was approximately two variable loci per 100 loci per 10 years. Clustering analyses based on similarity estimates showed that the fiber cultivars were more related (or similar to each other) and were classified as a homogeneous group. All of the linseed cultivars were clustered in diverse groups with the nine landrace accessions. Implications of these findings for flax breeding and germplasm management are discussed.

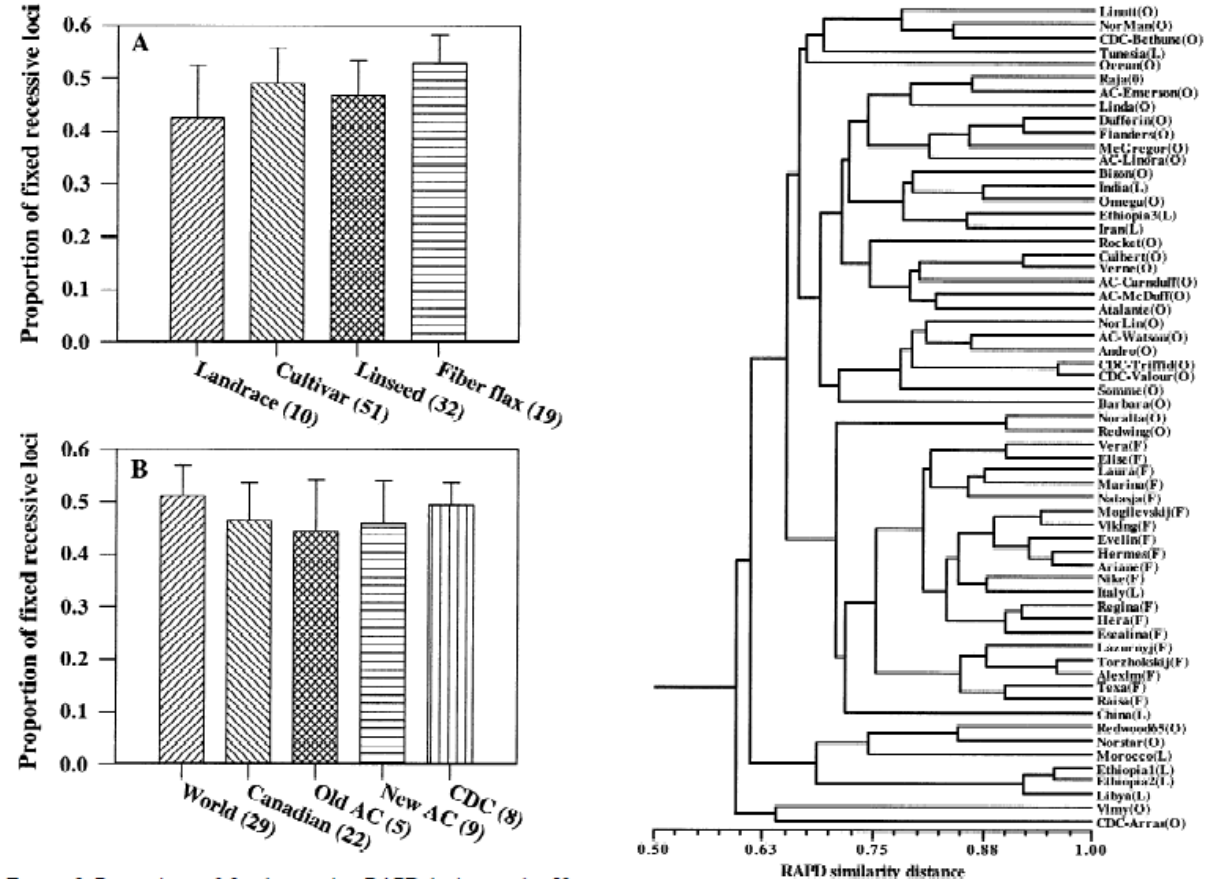

Fig. 40 ***LEFT: Proportions of fixed recessive RAPD loci over the 53*** **variable RAPD loci observed for various groups of flax accessions. Their standard errors are shown in bar. The number following the group label is the number of accessions used for the group.** ***RIGHT: Genetic relationships of all the 61 flax accessions re*****flected in RAPD similarity distance. The letters (O, F, L) following the accession names stand for oil, fiber, landrace flax, respectively. From (Fu et al., 2002)**

2. Biosafety considerations

2.1. Gene flow

L. usitatissimum and *L. bienne* can freely produce fertile F1 *hybrids* (Green, 1983). *However, L. usitatissimum* × *L. catharticum* are strongly isolated (Green, 1983). (Seethara.A, 1972) examined crossing relationships between wild *Linum* taxa and cultivated *L. usitatissimum*. Levels of hybrid seed set were typically high and normal meiotic pairing was observed in most F1 hybrid combinations. Interfertility of cultivated flax with the native *L. monogynum* (New Zealand) is unknown.

This view is differentiated by (Seethara.A, 1972):
Twelve interspecific hybrids obtained by crossing 9 different 30-chromosome species of the genus Linum were studied in detail for important morphological characters like, tillering, height, secondary branching, oil content, rust resistance, pollen and seed Sterility in the hybrids. L. strictum appeared to be important from the viewpoint of improvement of the cultivated linseeds with regard to number of tillers. The oil content in different species ranged from 24.6 % in L. palliscence to 37.1 % in L. angustifolium.The fact that L. angustifolium tops the list of the wild species in oil content seems to lend support to the view that it is one of the progenitors of L. usitatissimum.
All the species under study were found resistant to all the locally prevalent seven Indian races of rust. They can be sources of rust-resistant genes to the cultivated linseeds. The hybrids showed varying degrees of pollen and seed sterility in spite of their chromosomes pairing and separating regularly, Studies are needed in order to understand the real potential of hybridization in the field. *On the other hand one has to understand that Linum usitatissimum is grown in most cases in the absence of interfertile close relatives.*

The collection and study of wild relatives is an integral part of any sound breeding programme. such investigations throw some light on the usefulness of these materials as gene sources for breeding. The first report of successful interspecific hybridization came from TAMME(1923). She obtained fertile hybrids between *L. usitatissimum* and *L. angustifolium* and the hybrid was intermediate between the parents. ROGACH (1941) crossed L. *Sibericum* with *L. alpinurn,* L*. narbonense* with *L. austriacum* and the hybrids obtained were highly sterile. RAY (1941) also attempted interspecific hybridization between 10 species and only hybridization between L. *usitatissimunz* and *L. angustifolium* was successful.
In the study o 12 (Seethara.A, 1972) interspecific hybrids involving 9 species were studied in detail for the important morphological characters of diagnostic value. Considerable diversity was observed between different species for different morphological characters like plant height, number of primary tillers, position and number of secondary branches, 1000-grain weight and oil content in the seed.

Regarding oil content in the wild species, it was generally lower than in the cultivated types. Amongst the wild species the maximum oil content was in L. *angustifoliunz* with 37.1 %. Incidentally this species has maximum 1000-grain weight also amongst the wild species. According to DE CANDOLLE (1904) and HEER (1865, 1872) the cultivated linseeds are said to have arisen from L. *angustifolium* presumably by human selection. The occurrence of genes for oil production in L. *angustifolium* provides some additional evidence of its ancestry for this school of thought. In view of the low oil content of the wild species they do not seem to be an important source of genic material. But their utility in improving oil quality may prove to be of value as PLESSE(R19S6 6) observed variation in the fatty acid composition of the oil of different species. The wild species seem to be also important as gene sources for rust resistance since they possess resistance for all the prevailing races of India.
The interspecific hybrids were generally intermediate for most of the morphological characters studied. However some of them exhibited hybrid vigour for characters like height, number of tillers etc. All the hybrids expressed partial pollen sterility, which was reflected in reduced seed fertility. An attempt was also made to cross the species with varying chromosome numbers with the cultivated species but without any success. SIZOVA(1958, 1961) studied the process of fertilization in some interspecific crosses with varying chromosomes. She observed partially developed embryos and attributed the incompatibility to somatoplastic sterility. It may be possible by embryo culture to overcome this difficulty, and secure fertile hybrids which may be useful not only in transferring some of its useful genes into the cultivated species but also in establishing interrelationships.

More literature references related to taxonomy and biosafety:
(Blaringhem, 1921; BretagneSagnard et al., 1996; Burdon et al., 1999; Chaudhuri & Sen, 1976; Chen & Dribnenki, 2002; Cross et al., 2003; Diederichsen, 2001; Dubey & Singh, 1966; Ferguson et al., 1997; Foster et al., 1998; Friedt et al., 1995; Gill & Yermanos, 1967a, b; Gorshkova et al., 1998; Gorshkova et al., 2003; Gorshkova et al., 2000; Himmelsbach et al., 1998; Jarosz & Burdon, 1991; Jeanmonod & Schlussel, 2003; Kearns & Inouye, 1994; Lei et al., 2003; Lisson & Mendham, 2000; McDougall et al., 1992; Muravenko et al., 2003; Murray, 1980; Ockendon, 1968, 1971; Oostdam & vanderPlas, 1996; Roberts & Pryor, 1995; Rogers, 1982; Salonen & Lammi, 2001; Salonen & Suhonen, 1995; Seethara.A, 1971; Seethara.A & Srinivas.D, 1972; Srinivas.D et al., 1972; Stegnii et al., 2000; Sundback et al., 2003; Sylven, 1925; Webb, 1964; Yermanos & Gill, 1969; Yilmaz & Kaynak, 2006a, b)

The keyword Linum yields some 1482 references in the Web of Sciences
http://www.botanischergarten.ch/EPOBIO-Linum/Bibliography-LINUM-WOS-20070121.pdf

Linum, flax

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)**	**0,1**
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)**	**2**
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)**	**0,1**

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)**	**0,1**
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)**	**2**
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)**	**0,1**
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)**	**?**

CSF	**Propensity for successful seed- and tiller mediated gene flow from commercial crop to feral**	
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)**	**0,1**
CGC3	**Does crop produce volunteers and/or seed deposits and pose an environmental threat ? (0/1)**	**?**

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)**	**2 (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**

Miscanthus

1. Taxonomy

Fig. 41 Emily Heaton, researcher at the University of Illinois, presents a field of Miscanthus x giganteus, the ideal biofuel plant. Internet-source: www.cndwebzine.hcp.ma/cnd_sii/article.php3?id...

Fig. 42 Miscanthus x giganteus, inflorescence

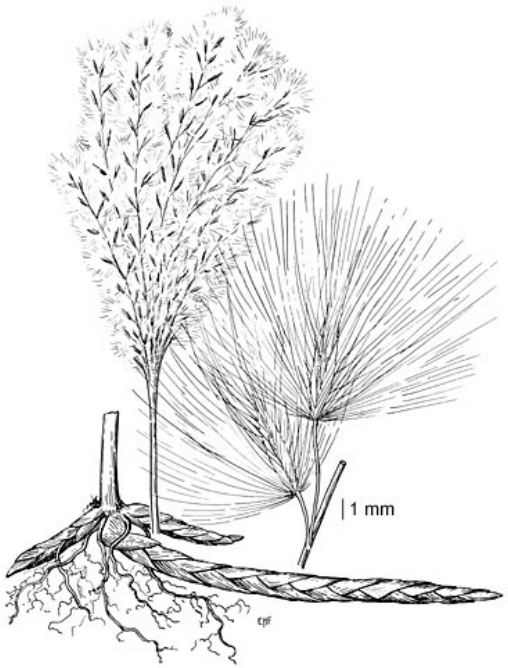

Fig. 43 Miscanthus x giganteus, and Rhizomes

Synopsis after (Greef & Deuter, 1993), compared to selected genera:

family	*Poaceae*
subfamily	*Panicoideae*
tribe	*Andropogoneae*
subtribe	*Saccharineae*
	Saccharininae
genus	*Imperata*
	Erianthus
	Saccharum
	Miscanthidium
	Miscanthus
	Eulaliininae
genus	*Eulalia*
	Microstegium

Fig. 44 Taxonomy of the genus Miscanthus compared with selected genera, after Pilger 1954, from (Greef & Deuter, 1993), Tab. 1.

According to (Greef & Deuter, 1993) the genus Miscanthus is originated to Southeast Asia. It shows a geographical distribution from tropical, subtropical and warm temperate parts of Southeast Asia to the Pacific Islands. M. sinensis and M. sacchariflorus in Japan

(Mutoh et al., 1985) and M.floridulus in Taiwan and New Guinea (Chou & Ueng, 1992) are known to form the most common perennial grassland communities. They are dominants of major disclimax communities as these occur in semi-natural grassland types in Japan due to human impact, such as burning and/or mowing (Mutoh et al., 1985). The generic name Miscanthus was established in 1855 by (Andersson, 1856) . Its species have been considered by a number of taxonomists describing a few species such as Imperata, Erianthus, Saccharum and Eulalia. The genus Miscanthus is a member of the tribe Andropogoneae, together with Saccharum, sugar cane. Following the taxonomy of (Pilger, 1954), the genus Miscanthus is also included in the subtribe Saccharineae (fig. 4).

The syntaxonomy of the genus is complicated due to polymorphism. (Adati & Shiotani, 1962) proposed an amphidiploid type of origin based on cytological and morphological studies. The authors pointed out that the high basic chromosome number of 19 suggests that Miscanthus has been derived probably from two ancestors, one with 10 chromosomes in Saccharininae and the other with 9 chromosomes in Eulaliininae. (Li et al., 1961) investigations confirmed this; they found that species of Miscanthus are easily crossed with Saccharum species (and other related genera of the tribe Andropogoneae, see also (Amalraj & Balasundaram, 2005)), showing a few of the chromosomes from Miscanthus to be partially homologous to those ofSaccharrlm. The genus Miscanthus is included in the "Saccharum complex" (Saccharum, Erianthus, Sclerostachya, and Narenga) due the close relationship to Saccharurn (Daniels & Roach, 1987). In another study about variation of nuclear ribosomal DNA including many Saccharum species, one Erianthus and one Miscanthus species (not specified) it was found that Erianthus and Miscanthus shared a unique restriction pattern, which was not similar to Saccharum species (Al-Janabi et al., 1994; Glaszmann et al., 1997).

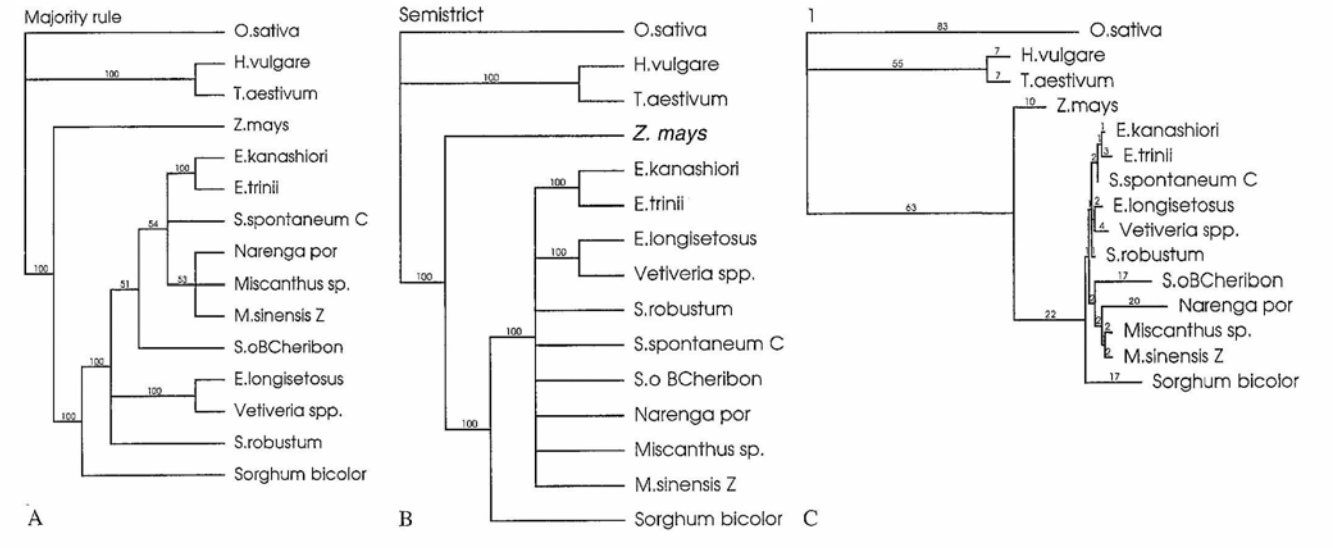

Fig. 45 A-C Phylogenetic hypothesis generated by analysis of *rbcLatpB* sequences. *A* Fifty percent majority rule of 79 equally parsimonious trees generated from analysis of 664 nucleotides and 55 insertion/deletion events scored as unordered binary characters (1,O); *numbers* on branches refer

to number of times (in percentage) in the 79 trees in which the bifurcation was supported. B Semistrict consensus of 79 trees, as in A. C Example of 1 of the 79 equally parsimonious trees, represented as a phylogram in which branch lengths (shown above lines) are proportional to genetic distances calculated in PAUP. In these trees, the following terminal taxa represent more than one accession: Z. *mays* (2 genotypes sequenced); *Saccharum robustum* = S. *barberi* = *S. edule* = *S. officinarum NG 5* 1-13 1; *S. officinarum* Black Cheribon = *S. sinense* from (Al-Janabi et al., 1994)

In their study on the phylogenetics of sugarcane (Saccharum officinarum L.) and its relatives (Al-Janabi et al., 1994) sequenced four loci on cytoplasmic genomes (two chloroplast and two mitochondrial) and analyzed mitochondrial RFLPs. Sequenced mitochondrial loci were nearly invariant within the Saccharum complex members, supporting conclusions drawn from the analysis of the chloroplast regarding the maternal lineages studied herein. In addition, one particular Miscanthus accession presumably of hybrid origin because of its high chromosome number, displayed the same mitochondrial type as Saccharum species, whereas Miscanthus sinensis showed a different type. This further substantiates the possibility of intergeneric hybridization in the wild, as now chloroplast and mitochondrial sequences of this accession have been shown to be the same as those of Sacharum, although we cannot exclude polymorphism within *Miscanthus* as an alternative explanation because of the limited number of accessions they studied.
A synoptic map of Miscanthus in relation to Sacharum has been published by (Hodkinson et al., 2002a)

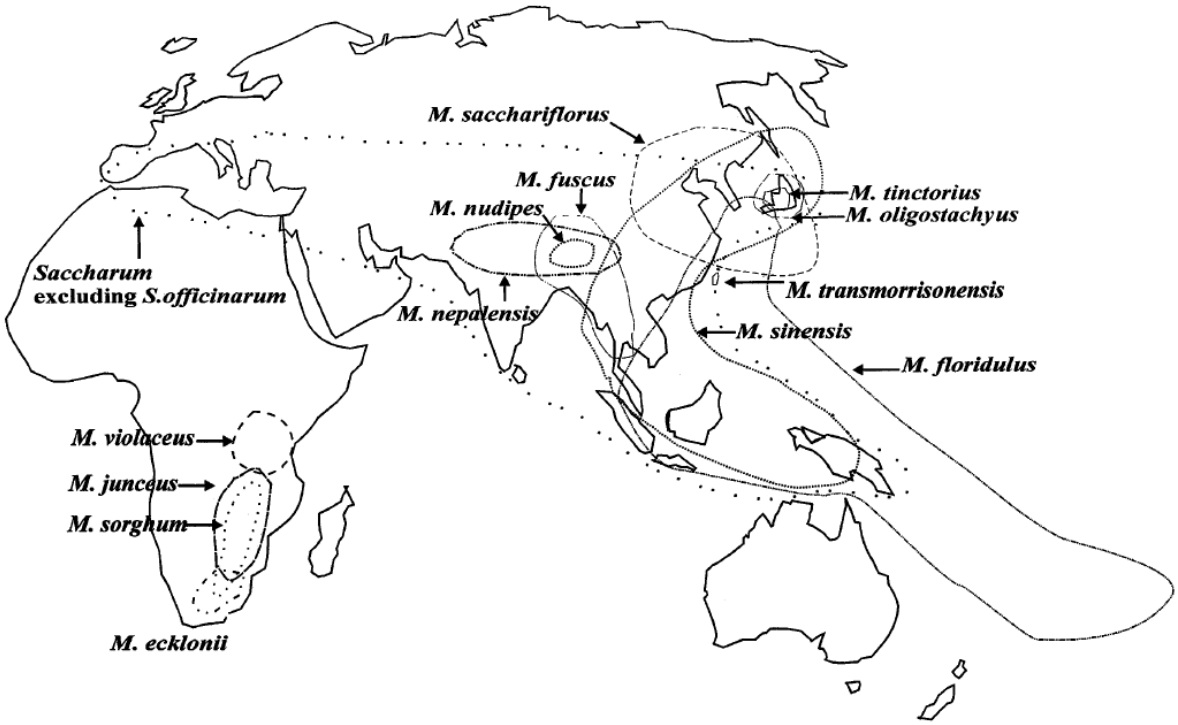

Fig. 46 Distribution of *Miscanthus* and *Saccharum sensu lato* species in the Old World. The major areas of distribution are shown by rings, but exclude occasional records from elsewhere. *Saccharum officinarum* (sugarcane) is not included because it has a widespread distribution due to cultivation. From (Hodkinson et al., 2002a)

Overall, even today the taxonomic status of Miscanthus is still in a state of flux, and little is known about the identity and interrelationships of its species. According to (Clayton & Renvoize, 1986), Miscanthus s.l. (in the wider sense) comprises approximately 20 species and appears well-defined morphologically. However, they also recognised that Eriochrysis, Eulalia, Imperata, Miscanthus, Saccharum and Spodiopogon form a closely knit group in which the phylogenetic relationships are unclear. *Saccharum* is considered by many as the closest relative of Miscanthus , and these two genera frequently hybridise (Sobral et al., 1994).

Some progress as been made by (Hodkinson et al., 2002a):
They can now recognise the distinction between Saccharum s.s., Miscanthus s.s., Ripidium (= Saccharum section Ripidium) and a newly defined Miscanthidium group, and there is evidence to support the monophyly of each of these. The other members of the “Saccharum complex” are more difficult to position. More studies are needed to find a better way of subdividing the remaining members of the Saccharinae (including the Saccharum complex) into genera or infrageneric taxa.

On the basis of the DNA sequence data Miscanthus s.l. and Saccharum s.l. are polyphyletic. Saccharum section Ripidium is separate from all other Saccharum species in their analysis and may be best treated as the genus Ripidium Trin. following (Grassl, 1972), see also (Besse & McIntyre, 1999; Besse et al., 1997).
Six Miscanthus species form a well-supported clade (group b; Fig. 2), and these have a unique basic chromo391 some number of 19. Miscanthus ´ giganteus Greef & Deuter ex Hodkinson & Renvoize (Hodkinson and Renvoize 2001), an allopolyploid hybrid of M. sinensis and M. sacchariflorus, would also form part of this group. The Himalayan species M. fuscus groups more closely with the African Miscanthidium and Saccharum contortum than with the southeastern Asian Miscanthus s.s. Miscanthus section Diandra of Lee (1964d), also found in the Himalayan region, and represented by M. nepalensis, does not group with any member of Miscanthus s.l.. Recently, its species have been recognised as a separate genus, Diandranthus (Trin) L. Liu, and our molecular analysis would add weight to this separation.

This is well demonstrated by a parsimony tree for Miscanthus, Saccharum and related species for the combined data matrix of ITS and the trnL-F intron and spacer regions of plastid DNA

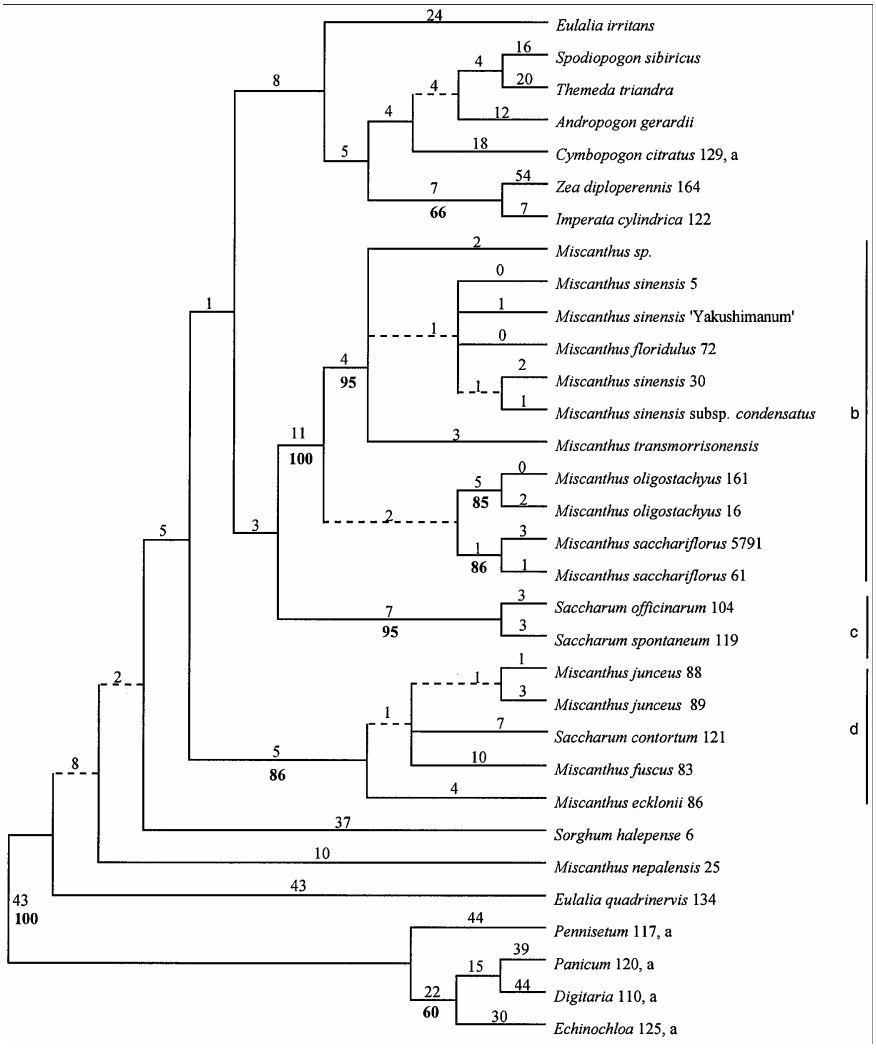

Fig. 47 Parsimony tree for Miscanthus, Saccharum and related genera for the combined data matrix of ITS and the trnL-F intron and spacer regions of plastid DNA. One of 32,039 equally most parsimonious trees. Length = 614, CI = 0.69, RI = 0.68. Values above branches are steps. Numbers below branches are bootstrap percentages above 50%. Groups found in all shortest trees are indicated by solid lines (groups not found in all shortest trees by a dotted line) from (Hodkinson et al., 2002a)

On the species level, it is worthwile to name the two most important taxa worldwide which are cultivated worldwide, also of mounting importance in Europe:
According to (Hodkinson et al., 2002b), most research investigating the productivity and ecological potential of Miscanthus has centered on two taxa: Miscanthus sacchariflorus (Maxim.) Benth. & Hooker, a species from northern China and Japan, and M. x giganteus Greef & Deuter ex Hodkinson & Renvoize (Hodkinson & Renvoize, 2001), a putative hybrid between M. sacchariflorus and M. sinensis Anderss. (Adati & Shiotani, 1962; Linde-Laursen, 1993). Miscanthus x giganteus Greef & Deuter (Greef & Deuter, 1993) but the latter name is invalid under the rules of the International Code of Botanical Nomenclature because the diagnosis and the description were in English and no type specimen was designated (Greuter et al., 2000). Miscanthus x giganteus is also incorrectly known as M. giganteus or M. sinensis ‘Giganteus’ and is often mistaken for M. sacchariflorus, see (Hodkinson & Renvoize, 2001) and (Hodkinson et al., 2002c) for

more details. The agricultural community is fully aware of the hazards to rely on single clones for high yields because of higher susceptibility to pests and diseases. Also it will be more difficult to adapt those clones to local climatic and edaphic conditions.
More attempts should be made for those reasons to broaden the genetic basis of Miscanthus x giganteus – this would mean that more efforts have to be made to study the genetic diversity of the genus.

The most detailed genetic analysis tree has been published by (Hodkinson et al., 2002b), but the figure 4 therein p. 633 is not availably for copy – paste.

2. Reproduction biology

2.1. Apomixis in general

Apomixis, clonal reproduction, clonal reproduction via seed is well documented in more than 40 angiosperm families (Richards, 1986). Devoid of genetic recombination, processes usually result in genetic uniformity within populations or even across populations within species. However, we need to know more about the embryological details and totipotence of the reproductive cells (Czapik, 1999).
General comments from a recent review of (Ozias-Akins, 2006): Asexual reproduction through seeds, or apomixis, is widespread in angiosperms, although does not happen frequently. It occurs in no major crop plant, but its deployment in major crops would afford advantages for breeding and maintenance of hybrid genotypes. Deployment is still a long-term goal, however, since the genetic mechanisms underlying apomixis in nature have not been determined nor has the isolation of apomictic mutants in sexual plants been achieved. Nevertheless, an increasing intensity of research toward these goals over the last decade has greatly expanded our knowledge of genome structure and gene expression in naturally occurring apomicts and female gametophyte development in sexual plants. A common working hypothesis is that apomixis is a "deregulation" of sexual processes and is increasingly supported by gene expression data. Nevertheless, the search for a unique trigger that initiates apomictic development still cannot be disqualified. Further characterization of female gametophyte-related genes and genomes of apomicts and model sexual plants will be fruitful for identifying overlaps in developmental networks. Another recent review comes to the same conclusion (Kumar, 2006), both reviews contain the recent literature on apomixis.

In recent years, some authors and research teams have realized that apomixis could play an important role in the mitigation of unwelcome gene flow in transgenic crops: (Daniell, 2002; Grossniklaus et al., 2001; Koltunow & Grossniklaus, 2003; Ramulu et al., 1999; Spillane et al., 2004; van Dijk & van Damme, 2000). It should also be noted, that a leading group of apomixis researchers came up with a ‚Bellagio Declaration' (Bellagio Apomixis Declaration, 1998): The undersigned urge widespread adoption of the principle of broad and equitable access to plant biotechnologies, especially apomixis technology, and we encourage the development of novel approaches for technology generation, patenting, and licensing that can achieve this goal.

2.2. Apomixis in Miscanthus

Miscanthus Andersson is typical of many dominant grasses of eastern Asia, being wind-pollinated and usually monoecious. Vegetative propagation via rhizomes is the most common means by which patches are able to expand.
In addition to their clonal nature, sterile pollen has been well documented in M. *sinensis* var. *condensatus* (Adati & Shiotani, 1962). This taxon should probably be named Miscanthus x giganteus, see discussion in chapter on taxonomy. In contrast, viable pollen, which is capable of being carried over long distances, and normal fertilization occur in most other *Miscanthus* taxa. Seed dispersal, however, is constrained by seed weight but can reach up to *5* m (Chou & Lee, 1991). A previous RAPD investigation of Miscanthus floridulus (Labill) Warb., another dominant lowland species, revealed significant genetic differentiation and variability among populations (Chen et al., 1993). Among the many biological factors affecting the evolution of populations, the mating system plays a critical role in determining heterogeneity, heterozygosity and outcrossing levels in populations. Apomixis is believed to be a force that reduces heterogeneity and moves the genetic composition towards fixation (Weising et al., 1995).

3. Biosafety considerations for Miscanthus

3.1. General remarks

The Challenge is obvious, realizing plans to produce energy with Miscanthus in Europe: (Clifton-Brown et al., 2004; Lewandowski et al., 2000): Miscanthus, a genus with C4 photosynthesis and a native of E. Asia, has good potential as a biomass energy crop (Jones & Walsh, 2001). Field trials in Europe during the last 15 years with the sterile, triploid hybrid M. x giganteus (Hodkinson et al., 2002a; Hodkinson et al., 2002b; Hodkinson et al., 2002c) have shown that this genotype can, from its second to third year following establishment, produce annual harvestable yields from 10 to 40 t dry matter (DM) ha^{-1} yr^{-1} (Lewandowskia et al., 2000). Reports of plant losses of this hybrid during winter in some climates have led to field trials to test other hybrid Miscanthus genotypes. However, where no over-wintering problems have occurred, M. x giganteus has proven to be among the most productive of all genotypes tested to date (Clifton-Brown et al., 2001). It produces exclusively sterile pollen and thus does not pose any biosafety problems through pollen mediated gene flow.

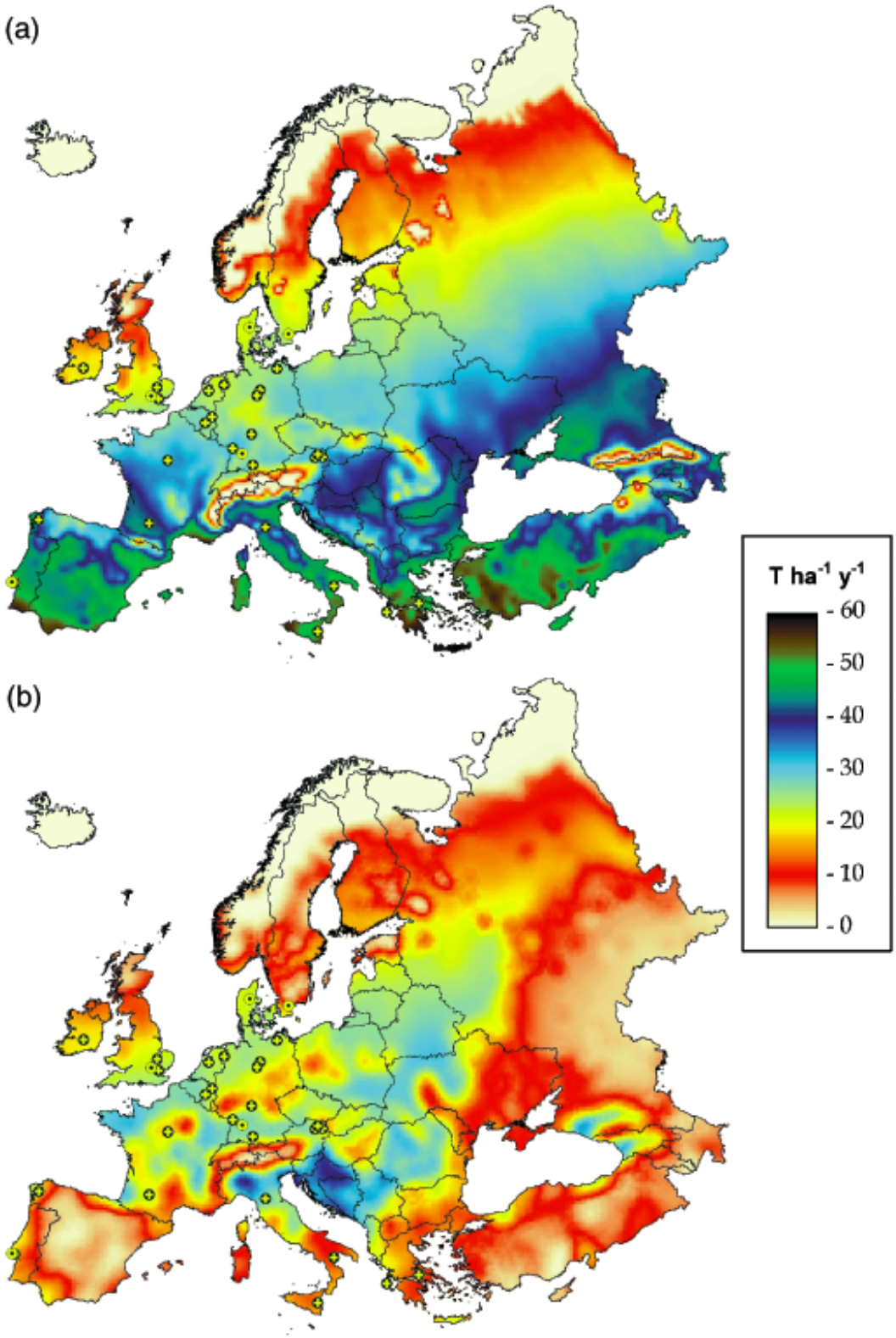

Fig. 48 Modelled yields of Miscanthus in Europe at the end of the growing season. (a) Potential non-water-limited yield and (b) rainfed yield (t ha_1). Positions of EMI field trials are indicated by + and other field trials by o .

It will be important to test apomixis of the traits used for transformation for the following reasons: Miscanthus x giganteus is the crop of choice, but although nomenclatural problems seem to be clarified, it is well known from many other crop cases that even in classic and well known core collections of international organizations one will always be confronted with erroneous labels etc.

3.2. Biosafety evaluation of the field release of transgenic poplars according to the Dutch-Swiss-Irish method

The Dutch-Swiss-Irish method of classification of risks is based on several previous papers from Holland and Switzerland: Based on the two first publications (Frietema, 1994, 1996) a Swiss research group developed the evaluation system further on: (Ammann & Jacot, 2003; Ammann et al., 1999; Ammann et al., 1996, 2000, 2003). A latest improvement has been achieved by (Flannery et al., 2005), presenting an extended evaluation scheme, including now also seed propagation. In this study, we extend it again with vegetative propagation potential, in order to adapt the system to the reproduction biology characteristics of Miscanthus.

Details and examples of the use of the 4 original Strands are given in (Flannery et al., 2005),

In this study, we extend the above described 4 strands again with vegetative propagation potential (tillering now additionally included in the evaluation lines for the seed and tuber mediated strands of CSV and CSF), in order to adapt the system to the reproduction biology characteristics of poplars and Miscanthus and other crops like Sorghum.

Two new strands are also added:
CGC: Consequences of Gene Flow and
CMG: Mitigation Gene Flow crop to crop, crop to wild relative and crop to feral

In summary, the following assessment of potential gene flow has been made on the system described above, extended with two new strands:

Miscanthus, Maiden Grass, apomictic traits

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)	0
CPW2	Is there a probability that the crop will flower and produce viable pollen during its cultivation? (0/1)	0
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)	0
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)	0
CPW5	If fertilization is achieved by the deposited pollen, will a viable F_1 hybrid individual establish itself? (0/1)	0

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)	0
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)	0
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)	-
CPC4	If fertilization happens, will a viable F_1 individual be established itself from hybrid seed in absence of mechanical/chemical control? (0/1)	-

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)**	**-**
CSV2	**Post-harvest, will the seed survive and germinate within the confines of a managed field? (0/1)**	**-**
CSV3	**Will the volunteer develop into a viable individual? (0/1)**	**-**

CSF	**Propensity for successful seed- and tiller mediated gene flow from commercial crop to feral**	
CSF1	**Does the crop produce seed during its cultivation? (0/1)**	**0**
CSF2	**Following transfer from cultivation site: will wayward seed survive and germinate? (0/1)**	**0**
CSF3	**Will the resulting individuals establish into a viable feral population? (0/1)**	**0**

CGC Consequences of Gene Flow

CGC1	**Does crop mix with related crop traits and jeopardize harvest value ? (0/1)**	**0**
CGC2	**Does crop mix with wild relatives or feral traits and pose an biodiversity threat ? (0/1)**	**0**
CGC3	**Does crop produce volunteers and/or seed deposits and pose an environmental threat ? (0/1)**	**0**

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)**	**1 (J)**
CMG2	**Mitigation with molecular safety measures: none, if strictly apomictic seed sterile traits are chosen apomixis AP, cytoplasmatic sterility CS, tandem construct TA, gene switching GS** **AP, TA, GS, (0/1)**	**AP, 1**

Nicotiana tabacum, Tobacco

1. Taxonomy

The family *Solanaceae* contains several well known cultivated crops such as tomato (*Lycopersicon esculentum*), eggplant (*Solanum melogena*), tobacco (*Nicotiana tabacum*), pepper (*Capsicum annuum*) and potato (*Solanum tuberosum*).

The natural distribution of the genus *Nicotiana,* family *Solanaceae,* is limited to America (75%), Australia and a few islands of the South Pacific (25%). The estimated 60 species of *Nicotiana* are classified into 14 sections based upon distribution and morphological and cytogenetic characteristics (Goodspeed, 1954). For example, inflorescence expression in section *Tomentosae* is in thyrse panicles except in *N. glutinosa.* The inflorescence of section *Alatae* shows monochasia, often extending dichasial forks. The traditional taxonomy is established primarily on the basis of morphology, distribution and cytology. However, factors like the environment, multigenic inheritance, or partial and complete dominance often confound the expression of a genetic trait (Tingey & Deltufo, 1993).

Molecular markers such as isozymes and restriction fragment length polymorphisms (RFLPs) have been extensively applied for genetic studies and plant breeding (Beckmann & Soller, 1983; Tanksley et al., 1989; Yang et al., 1992). Random amplified polymorphic DNA (RAPD) analysis is based on the amplification of random DNA segments with single primers of the arbitrary nucleotide sequence. The polymorphisms observed may result from nucleotide substitutions, insertions, or deletions. The major advantages of this analysis are that (1) the information of DNA sequence is not required, (2) the protocol is relatively easy to perform, (3) only a small quantity (ng) of DNA is needed, (4) a large number of samples can be processed simultaneously in a short period of time, (5) the technique can be applied to a broad range of species (Martin & Garcia, 1991; Welsh & McClelland, 1990) etc.

To apply RAPD for analysis of phylogenetic relationships, nine species from two sections of *Nicotiana (Tomentosae and Alatae) were examined. Nicotiana sylvestris* is considered a member of *Alatae* (Goodspeed, 1954). However, Kostoff (1943) observed that F1 hybrids of *N. sylvestris* and any other species in section *Alatae* had lower chromosome associations than F1 hybrids of N. *sylvestris* and any member of section *Tomentosae* had.

Hence, he regarded *N. sylvestris* as a member of section *Tomentosae.* In this study, we performed RAPD assay to analyze the phylogenetic relationships among 18 genotypes of *Nicotiana* and determine the taxonomic position of *N. sylvestris.*

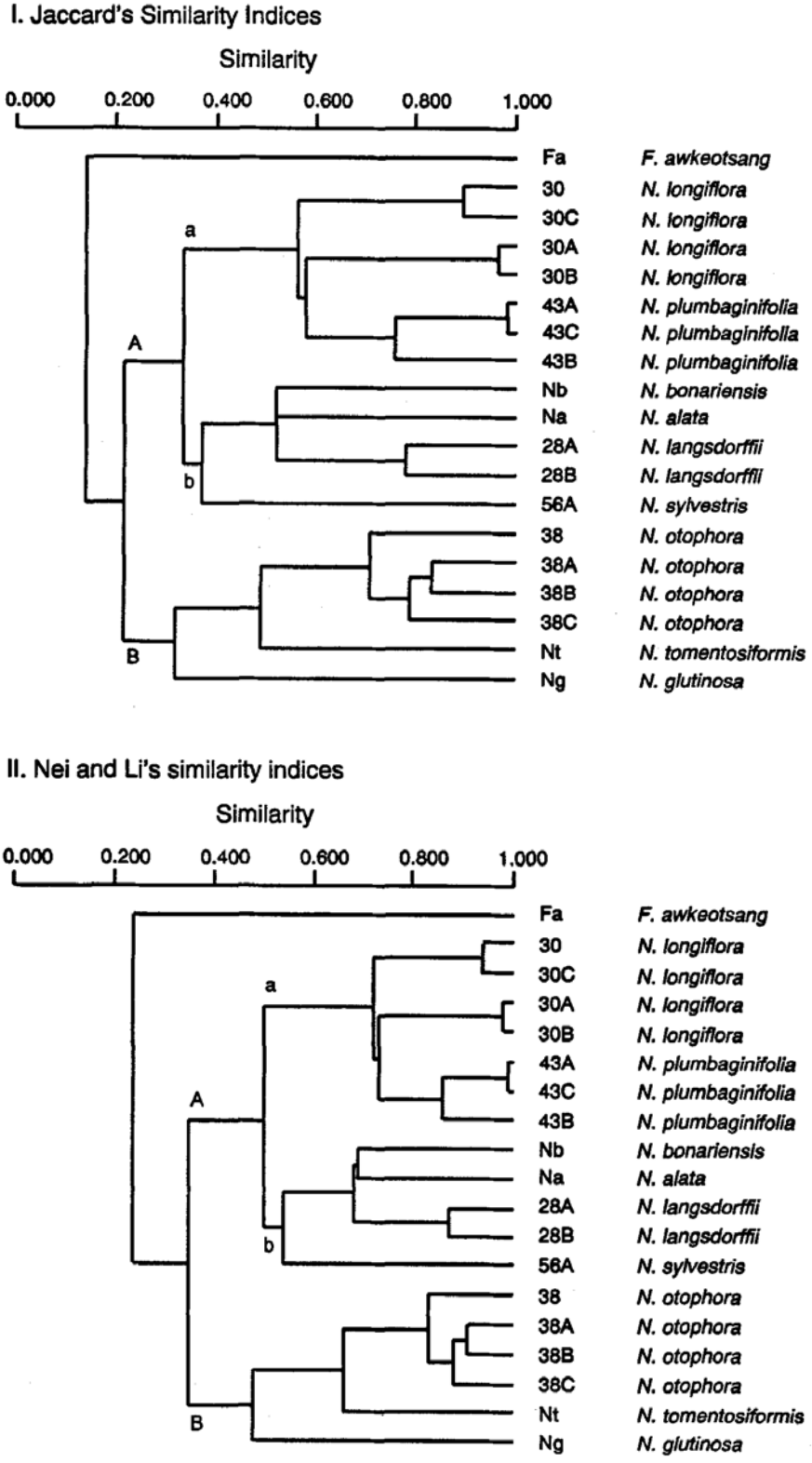

Fig. 49 Dendrogram constructed using the UPGMA based on Jaccard's similarity coefficients (I) and Nei and Li's similarity coefficients (11) illustrating the genetic relationships among 18 genotypes of *Nicotiana and Ficus awkeotsang.* Relative lengths indicate similarity indices. The abbreviations of samples are the same as those indicated in Table 1 below, from (Yu & Lin, 1997)

Table 1. The genotypes in *Nicotiana* used for RAPD analysis

Section	Species	Acc. no.	Chromosome no. (2n)	Source
Alatae				
	N. longiflora (Nlo)	30	20	USDA
		30A	20	USDA
		30B	20	USDA
		30C	20	USDA
	N. plumbaginifolia (Np)	43A	20	USDA
		43B	20	USDA
		43C	20	USDA
	N. bonariensis (Nb)		18	TTRI
	N. alata (Na)	3	18	USDA
	N. langsdorffii (Nla)	28A	18	USDA
		28B	18	USDA
	N. sylvestris (Ns)	56A	24	USDA
Tomentosae				
	N. otophora (No)	38	24	USDA
		38A	24	USDA
		38B	24	USDA
		30C	24	USDA
	N. tomentosiformis (Nt)		24	TTRI
	N. glutinosa (Ng)		24	TTRI

Fig. 50 The genotypes in *Nicotiana* used for RAPD analysis, from (Yu & Lin, 1997)

In accordance with the classification of (Goodspeed, 1954), the 18 genotypes of *Nicotiana* were divided into two clusters (A and B) based on the phylogenetic tree constructed (Figure 2), cluster A belonging to section *Alatae* and cluster B belonging to section *Tomentosae.* The results of (Yu & Lin, 1997) could also suggest that the dendrogram divides these species into three clusters B, b and a. In that case section *Alatae* is divided into two clusters with *N. longiflora* and *N. plumbaginifolia,* the two species of 10-paired chromosome, belonging to cluster a and the other four species in *Alatae* belonging to cluster b. *Nicotiana longiflora* and *N. plumbaginifolia* are almost identical in external morphology, chromosome number and karyotype (Goodspeed, 1954). The species of 9-paired chromosome, *N. alata* and *N. langsdorffii* are located in subcluster b of cluster A. In cluster B the similarity coefficient between N. otophora and N. tomentosiformis is higher (0.497 to 0.511 for Jaccard's similarity coefficients and 0.

664 to 0.677 for Nei and Li's similarity coefficients) than that between *N. otophora* and *N. glutinosa* (0.323 to 0,335 for Jaccard's similarity coefficients and 0.488 to 0.502 for Nei and Li's similarity coefficients). *Nicotiana otophora* and *N. tomentosiformis* are two of the three core species in section *Tomentosa* and exhibit affinity in external morphology.

However, *N. glutinosa* is more or less marginal species in section Tomentosa. Nicotiana sylvestris, which is a member of section *Alatae* according to the classification of (Goodspeed, 1954), was regarded as a member of section *Tomentosae* according to cytogenetic studies (Kostoff, 1943). *N. sylvestris* has 2n=24 chromosomes as in species of section *Tomentosa.* This data suggest that *N. sylvestris* is at least basal to the clade of section *Alatae.* The results from the RAPD analysis conform to the classification of (Goodspeed, 1954) and coincide with a previous study of physical maps of some *Nicotiana* chloroplast DNA (Yang et al., 1992). The size and basic structure of the chloroplast DNA from *N. sylvestris* were found to be almost identical to that from N. otophora and N. plumbaginifolia; however, the wealth of restriction site variation in N. *otophora* chloroplast DNA compared to N. sylvestris and N. plumbaginifolia suggested that N. sylvestris and N. plumbaginifolia were distantly *related to N. otophora.*
PCR that is used to produce informative amplification products often produces artifactual products as well. To eliminate RAPD artifacts, we carried out at least two replicates and analyzed only those bands that were reproducible. However, discarding faint or inconsistant bands could introduce false negatives into the data. It is *also* possible Li's coefficient is recommanded as it displays less percent bias than Jaccard's coefficient (Lamboy, 1994a, b, c). Based on our analyses, the values of Nei and Li's coefficients are always higher than that of Jaccard's coefficients for each pair of data. The difference in the values might be resulted from the bias included in Jaccard's coefficient. Our results showed the advantage of Nei and Li's coefficient for computing genetic similarity coefficients for closely related species.
On the basis of the dendrogram constructed with the similarity coefficients generated from RAPD markers, the 18 *Nicotiana* genotypes were divided into two sections, *Tomentosae* and *Alatae,* in accordance with the classification of (Goodspeed, 1954). This study demonstrated that RAPD assay is a rapid and sensitive technique for identifying phylogenetic relationships at the interspecific and the intraspecific levels in *Nicotiana.*

2. Biosafety considerations

Many Nicotiana species are inbreeders, but this also depends on many factors.

(Sime & Baldwin, 2003) undertook a study on opportunistic out-crossing in Nicotiana attenuata, a predominantly self-fertilizing native tobacco:
Background: Although *Nicotiana attenuata* is entirely self-compatible, chemical and other floral traits suggest selection for the maintenance of advertisement for moth pollinators.

Results: Experimental exclusions of pollinators from plants with emasculated flowers in natural populations in southern Utah during an outbreak of the hawkmoth *Hyles lineata* revealed that 24% of the seed set could be attributed to insect pollination, and eliminated wind pollination and apomixis as contributing to seed set. Hence these moths can mediate gene flow when self-pollen is unavailable. To quantify gene flow when self-pollen is available, plants were transformed with two marker genes: hygromycin-B

resistance and β-glucuronidase. The utility of these genetic markers to measure gene flow between plants was examined by mixing pollen from plants homozygous for both genes with self-pollen in different ratios and hand-pollinating emasculated flowers of plants growing in a natural population. The proportion of transformed seeds was positively correlated with the amount of transformed pollen applied to stigmas. In glasshouse experiments with the hawkmoth *Manduca sexta* and experimental arrays of transformed and wild-type plants, pollination mediated by moths accounted for 2.5% of the seed set.

Conclusions: Even though moth pollination is rare and highly variable for this largely selfing plant, *N. attenuata* opportunistically employs a mixed-mating system.

(Paul et al., 1995) concentrated on a study on gene dispersal via pollen in Nicotiana tabacum using introduced genetic markers: Full text copied

1. *Agrobacterium tumefaciens* was used to introduce two marker genes (kanamycin resistance and p glucuronidase) into tobacco.

2. These plants were grown in a series of field trials each consisting of a small plot of modified plants surrounded at various distances (1 m, 10 m, 20 m) by non-modified receptor plants.

3. Capsules from these receptor plants were harvested and samples of the seed were germinated on kanamycin-containing medium in laboratory conditions, as a screen for the presence of the resistance gene. Large populations of seed could be screened in this way.

4. Using these techniques, gene flow from the 'marked' plants could easily be detected. Although there were some differences in the absolute degree of gene dispersal in the different trials, there was an overall decline in transfer as distance from the marked plants increased.

5. The potential for the use of introduced genetic markers in the study of gene flow is discussed.

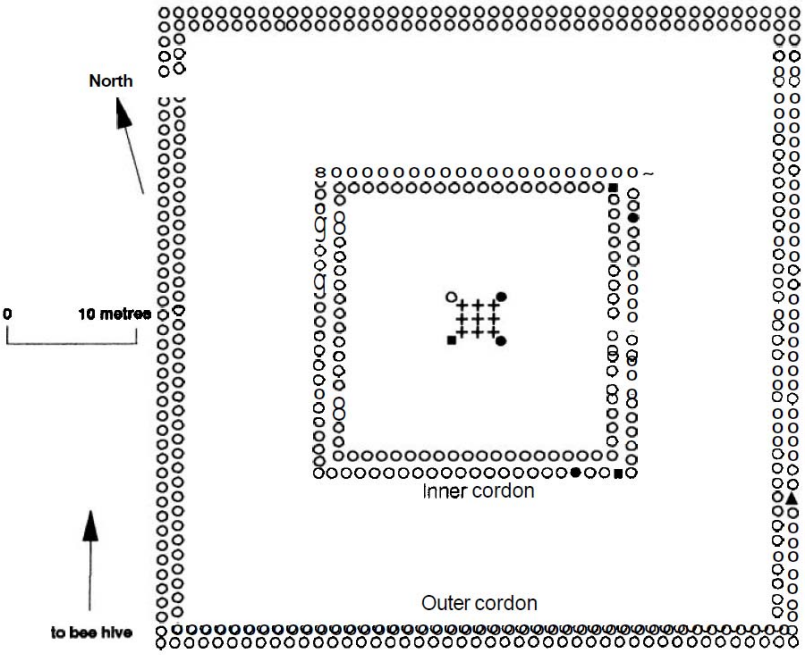

Fig. 51 Position of plants in Trial 1 that were found to have kanamycin-resistant progeny. +, nine genetically modified plants at the centre of the plot; ○, no kanamycin-resistant progeny; ●,l% or less of progeny kanamycin-resistant; ■, >%, but <*5%;* A, 5% or more.

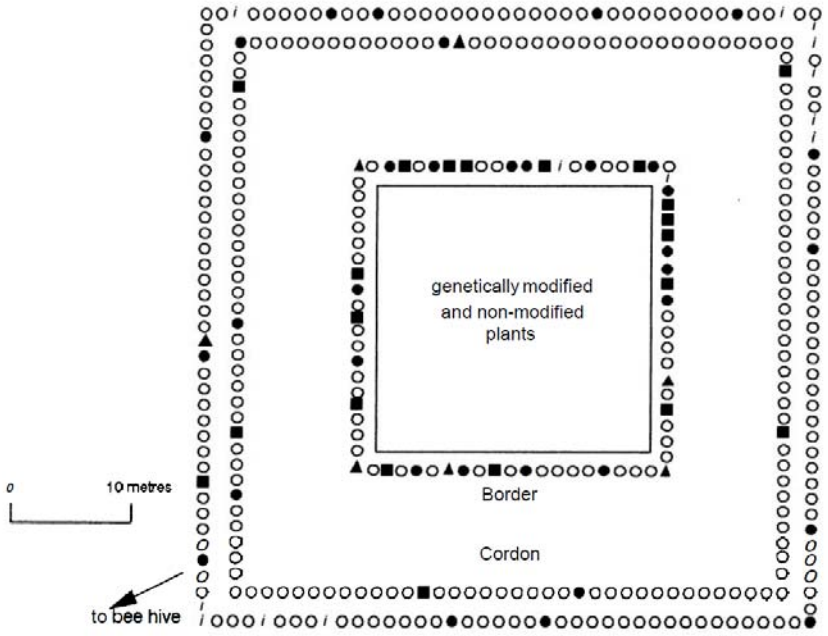

Fig. 52 Position of non-modified receptor plants in Trial 2 that were found to have kanamycin-resistant progeny. *i,* fungus-infected sample ○,no kanamycin-resistant progeny; ●, 1% or less of progeny kanamycin resistant;■.>,l% , but 4 % ; A , 5% or more.

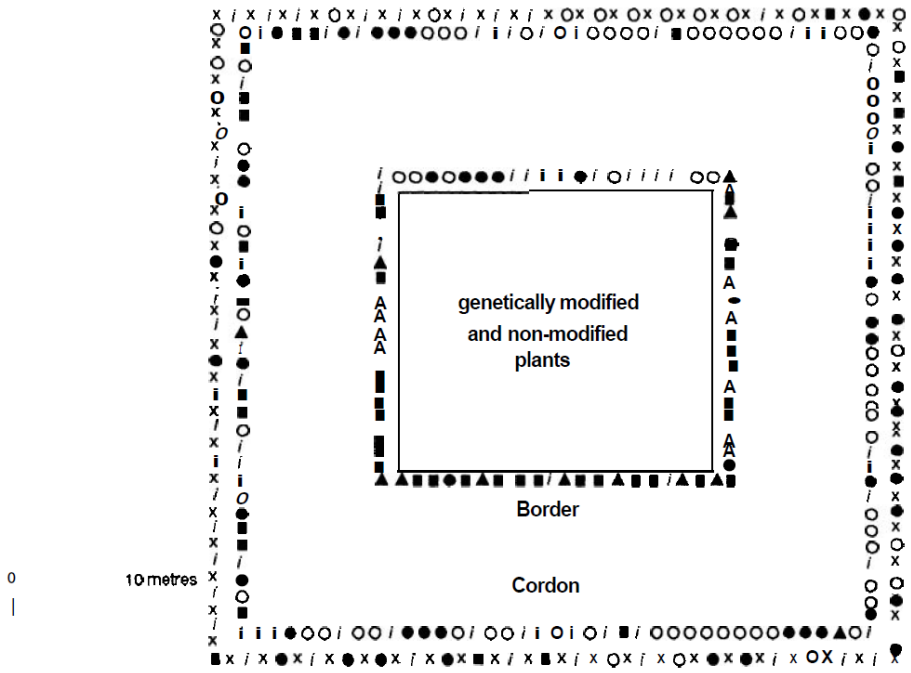

Fig. 53 Position of non-modified receptor plants in Trial 3 that were found to have kanamycin-resistant progeny, x, seed not tested; *i,* fungus-infected sample; ○, no kanamycin-resistant progeny; ●, 1% or less progeny kanamycin resistant; ■, >l%, but *5%;* A, *5%* or more.

Results from this series of field trials have confirmed the feasibility of screening progeny from non-modified receptor plants for presence of the marker trait introduced to the pollen donor plants by genetic transformation. Two other reported studies have also demonstrated the use of selectable, introduced markers (McHughen et al., 1990). In these field trials, with potato and flax, respectively, a low level of out-crossing from genetically modified plants was found, as expected for these crops. Previous studies of gene dispersal in various species have used markers such as flower colour (Kehr, 1973), chlorophyll deficiency (Stringam & Downey, 1978a, b) and isozyme patterns (Ellstrand et al., 1989; Jackson & Clarke, 1991) . These methods have the disadvantage that the pollen donors and receptors are usually different cultivars, with different morphology and development, which may influence pollination. By contrast, genetic modification allows the production of lines of isogenic plants, with and without the marker character. However, the transformation process can induce somaclonal variation or other differences and therefore examination of the modified plants is necessary to determine whether any unexpected effect had occurred.

Field trials have demonstrated that it is possible to produce modified plants that differ little in performance from nonmodified lines (Arnoldo et al., 1992; McHughen & Rowland, 1991). The lines used in the present study were examined in each generation to check that they showed no unexpected characteristics. In a field trial that included lines derived from this material, some reduction in vigour of modified as compared with non-modified plants was found (Caligari et al., 1993). For example, there was a 2-day delay in the time of first flowering in the modified line. Whether such a difference in development would

affect pollinator behaviour or other aspects of pollination would have to be determined by methods such as observation of bee preference. The production of modified plants has now been achieved in many herbaceous species (Raybould & Gray, 1993); so introduced markers might be used in the study of a wide range of species. However, to transform a species that has not been used previously may require considerable effort. The regulations for field work with genetically modified plants demand additional work; however, providing suitable conditions are arranged and appropriate marker genes are used, these regulations should not be prohibitive.

Introduced markers have the advantage that screening can be carried out at the seedling stage with a simple, unambiguous test. Also, molecular methods (such as the polymerase chain reaction), to confirm presence of the introduced genes, may be carried out on almost any plant material, including pollen and stored samples. More than one gene may be introduced (as in the present study) so that independent tests for hybridization can be carried out. Kanamycin resistance is a dominant character, and the screening used here did not distinguish between homozygotes and heterozygotes. For this distinction to be made, without continuing to the next generation, an examination at the protein level, e.g. by determining isozyme composition, would be required.

In the present trials, the fraction of resistant progeny from receptor plants fell with increasing distance from the modified plants. This would be expected, first because of dilution of the modified pollen as it was dispersed outwards from the small source area to the larger area occupied by the receptor plants; and secondly, because of dilution by non-modified pollen from plants in the cordon.

The distribution of plants producing resistant progeny appeared to be random, indicating that there was no directionality in pollination. It should be noted that the data report only the apparent extent of cross-fertilization of non-modified plants by pollen from modified plants; the concurrent cross-fertilization between non-modified plants was undetectable. Assuming that the fraction of modified pollen in the non-self pollen arriving at the stigma was proportional to the numbers of plants, the total level of out-crossing may be estimated. For this to be valid, the amount of pollen produced per plant should not be affected by the modification and there should be no discrimination in pollen movement or fertilization. In trials 2 and 3, the total rates of cross-fertilization of non-modified plants in the central area (considering all plants in the central area plus the border row as pollen donors) were 7.5 and 17.2, as compared with 4.1 and 9.4 for fertilization by the modified pollen alone. The overall hybridization level appears to have been high, in view of the self-pollinating nature of the tobacco plant, with the particularly high value in Trial 3 possibly being due to the more abundant pollinators. However, these results are similar to those from a study of tobacco carried out in the USA using a diseaseresistance marker, where the out-crossing rate of a plant varied from 0.3 to 3.7% in cultivar KY 16, and from 1 to 19% in Burley 37 (Litton & Stokes 1964) The fraction of resistant progeny would be equivalent to the fraction of modified pollen on the stigma if there were no difference in the competitive ability of the modified and non-modified pollen, and no discrimination between self- and cross-fertilization. The modification of the tobacco lines was not expected to affect fertilization and it was therefore assumed that the fraction of resistant seedlings was a true measure of out-crossing. It should be pointed out, however, that the screening detected only expression of the introduced character; thus, any hybrid progeny in which the gene is present; but not expressed would not have been detected. In order to detect the presence of recombinant DNA, polymerase chain reaction analysis could be used. However, this technique is expensive and complicated

in comparison with screening for expression of the introduced character. In such a study as that reported here, the particular method of sampling seeds for screening may be varied according to the information required. By combining the seed from many capsules, as in Trial 3, an estimate of the fraction of hybrid seed produced on a plant can be obtained.

This has the advantage that a large number of capsules can be sampled rapidly. However, the large number of samples with fungal contamination in Trial 3 may have been caused by combining seed from several capsules and so increasing the chance of including an infected capsule.

In trials 1 and 2, where seed from individual capsules was screened, additional information can be obtained on the fraction of capsules containing hybrids. In this way the minimum number of cross-pollination events can be estimated. For example, in trial 1, samples from the outer cordon showed that kanamycin-resistant progeny were present in one capsule from a single plant. Therefore, a minimum of one cross-pollination event had occurred. The maximum number of events is more difficult to estimate, since it is possible that pollen from more than one modified plant may fertilize a flower. The disadvantage of sampling individual capsules to assess overall frequencies is that a large number of samples must be screened because of the large variation between capsules.

The present series of field trials examined out-crossing over relatively short distances with a regular planting pattern in a closely controlled, agricultural environment. Extending these trials to examine dispersal over larger areas or in a non-agricultural environment would be more difficult. This is partly because the incidence of resistant seedlings was found to be highly variable, even with immediately adjacent plants, and over longer distances the modified pollen would be greatly diluted. Thus, very largescale screening would be required to detect such low frequency events. However, pollen dispersal to greater distances is possible since in previous studies it was concluded that pollen dispersal is leptokurtic; that is, much of the fall in cross-pollination occurs close to the source, but the rate of fall decreases with distance and a low frequency of cross-pollination may extend to long distances (Bateman, 1947a, b; Ellstrand & Devlin, 1989). Also, the relationship between cross-pollination frequency and distance from the pollen source is complex, since the frequency may depend on environmental factors, including the size of the pollen donor and receptor populations (Ellstrand & Devlin, 1989; Okubo & Levin, 1989). Indeed, in the present study, the fraction of resistant progeny differed between the three trials and may have been related to differences in population sizes, environments and pollinators. Whilst pollen dispersal over long distances may be detected, the significance of this in altering the composition of plant populations is difficult to predict and may be of limited impact because of factors such as selection against immigrant genotypes (Levin & Kerster, 1974). The use of modified plants offers an additional method for studying the influence of such factors on gene flow, as will be discussed in a future publication."

3. Mitigation

Experiments have been carried through by the Group of J. Gressel:

(Al-Ahmad et al., 2004): Some transgenic crops can introgress genes into other varieties of the crop, to related weeds or themselves remain as 'volunteer' weeds, potentially enhancing the invasiveness or weediness of the resulting offspring. The presently suggested mechanisms for transgene containment allow low frequency of gene release (leakage), requiring the mitigation of continued spread. Transgenic mitigation ™, where a desired primary gene is tandemly coupled with mitigating genes that are positive or neutral to the crop but deleterious to hybrids and their progeny, was tested as a mechanism to mitigate transgene introgression. Dwarfism, which typically increases crop yield while decreasing the ability to compete, was used as a mitigator. A construct of a dominant ahas R (acetohydroxy acid synthase) gene conferring herbicide resistance in tandem with the semidominant mitigator dwarfing Δ *gai* (gibberellic acid-insensitive) gene was transformed into tobacco (Nicotiana tabacum). The integration and the phenotypic stability of the tandemly linked ahas R and Δ gai genomic inserts in later generations were confirmed by polymerase chain reaction. The hemizygous semidwarf imazapyr-resistant TM T 1 (= BC 1) transgenic plants were weak competitors when cocultivated with wild type segregants under greenhouse conditions and without using the herbicide. The competition was most intense at close spacings typical of weed offspring. Most dwarf plants interspersed with wild type died at 1-cm, > 70% at 2.5-cm and 45% at 5-cm spacing, and the dwarf survivors formed no flowers. At 10-cm spacing, where few TM plants died, only those TM plants growing at the periphery of the large cultivation containers formed flowers, after the wild type plants terminated growth. The highest reproductive TM fitness relative to the wild type was 17%. The results demonstrate the suppression of crop–weed hybrids when competing with wild type weeds, or such crops as volunteer weeds, in seasons when the selector (herbicide) is not used. The linked unfitness would be continuously manifested in future generations, keeping the transgene at a low frequency.

4. Summary Gene Flow Nicotiana, draft

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)**	**2**
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)**	**2**
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	**Propensity for successful seed- and tiller mediated gene flow from commercial crop to feral**		
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)**		**2 (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**

Populus, Poplar

1. Some general remarks about regulation

Public-sector scientists need to play a serious, free role (Strauss et al., 2004)
The 'Monsanto model' exemplified the first phase of biotechnology development, where the private sector evaluated benefits for transgenic varieties internally, or dictated the terms for evaluation. Moreover, the focus has been on benefits for the company and farmer, rather than broad social and environmental values. Thus, it has been easy to demonize corporate, patent-dominated biotechnology as solely profit-driven. For this to change, much broader public sector participation is needed, both from a technical and ethical viewpoint.
As the Nuffield Foundation has shown best, there are strong ethical cases to be made for crop biotechnology http://www.nuffieldbioethics.org/gmcrops/index.asp
However, for reasons of credibility, corporations should not be the ones doing it.
Because the intellectual property constraints on transgenic products are being relaxed as patents expire, there may be increased public-sector release of regionally valuable transgenic varieties. This would also help to reduce the perception that biotechnology products only benefit corporations, and could allow many low-risk tree products to be released that would help to inform the public about the long-term efficacy of biosafety traits (e.g. sterility and dwarfism).

It will be important to establish in future project phases with concrete field releases for research and development some discursive decision making processes, in order to maintain and secure scientific argumentation. Scientific argumentation has some important prerequisites, such as discursive structures in decision making, which respect different kinds of knowledge, reduced hidden agendas, the rule that only participants are admitted who are part of the problem and last but not least obey to the principle of the symmetry of ignorance. This sounds as a strict recipe, but actually it is the contrary: the method of the systems approach secures maximum unobstructed space for a free debate: See (Ammann & Papazova Ammann, 2004) and cited literature within the text.

The author of this study was among the initiators of a new NGO which is helping to develop regulation on an international level (Cartaghena Protocol on Biosafety http://www.biodiv.org/biosafety/default.aspx within CBD) with a strong focus on *Science*.

Researchers of public institutions have now become active in the negociations for future improvement of the Cartagena biosafety protocol, which was shaped in recent years, solely focussing on the negative sides of biotechnology as a result of undue concentration on political issues, neglecting the rapidly developing knowledge on biosafety science. All necessary information, including sponsoring sources can be found on the website www.pubresreg.org. Among many other documents there are two which are interesting for this text: Guidelines, see www.pubresreg.org
specifically the proposed guidelines which can be found under , still in development

http://pubresreg.org/Members/Kim/working%20groups/biosafety%20protocol/CPB/Notificationguide

A PRRI working group also has submitted to the CBD a specific questionnaire on transgenic trees, see
http://files.pubresreg.org/PRRI%20submission%20on%20GM%20Trees%20%202006%20-%2008.pdf

2. Introduction

Populus has emerged with lots of justification as a model tree for transformation, the genus is often called the Arabidopsis of tree genetics. There are today some 3823 publications following a search in the Web of Sciences with Populus or Poplar in the title alone, more than 2000 from major countries in Europe, 600 from the last three years globally (Web of Science, 2006). There are many recognised species of poplars and these hybridise extensively in nature, with many more hybrids having been produced by controlled crossing. All of the species are deciduous, fastgrowing with a relatively short life span, are moisture loving, generally intolerant of shade, and are medium to large trees. Ease of propagation, speed of growth and hardiness has led to their popularity for use as ornamentals, windbreaks, large spacing and short-rotation “pulp” or “energy” plantations.

3. Taxonomy and its relation to biodiversity

Poplar species are members of the genus *Populus* L., in the family Salicaceae (willow family) and the order Salicales, which, together with the Flacourtiaceae and 29 other families, have been placed under the Malpighiales in the recent cladistic analysis of the angiosperms (Nandi et al., 1998) p. 153. A genus of deciduous trees (rarely semievergreen), it comprises aspens, poplars, and cottonwoods, having a wide natural distribution in the Northern Hemisphere and a small representation in tropical Africa. Various global classifications have been suggested, the most recent recognizing 29 species that are grouped under six separate sections (Eckenwalder, 1996).

The genus is traditionally subdivided taxonomically into sections. Five of these sections are widely recognised: *Turanga*, *Leucoides*, *Aigeiros*, *Tacamahaca*, and *Populus* (known synonymously as *Leuce*) (Zsuffa, 1975a). Some taxonomists have been inclined to add a sixth single-species section to resolve classification problems. The dispute over the

sectional classification of poplars is not settled; meanwhile, it is generally accepted that three of the sections are represented in Europe: *Populus*, *Aigeiros*, and *Tacamahaca* (Farrar, 1995; Franco, 1964; Krüssmann, 1985).
Four *sections* are listed in the Flora Europaea (Franco, 1964):
Populus, with Populus alba, canescens, grandidentata, tremula, x hybrida,
Tacamahaca, with Populus simonii, gileadensis, trichocarpa
Aigeiros, with Populus x berolinensis, nigra, x canadensis and several clones in cultivation, which arose independently at different places
Turanga, with Populus euphratica.
This is a total of 11 taxa on species level listed in the Flora Europaea, the major European species being Populus nigra, P. alba and P. tremula.

The figure from (Nandi et al., 1998) summarizes the many recent studies on cladistics on a molecular basis, which reshuffled important regions of the modern plant system. It will be important for future work on genetic engineering of new plant products to know the gene sequences and its functions, and more important: the genomic more or less close relationships to crops with lots of molecular data available.

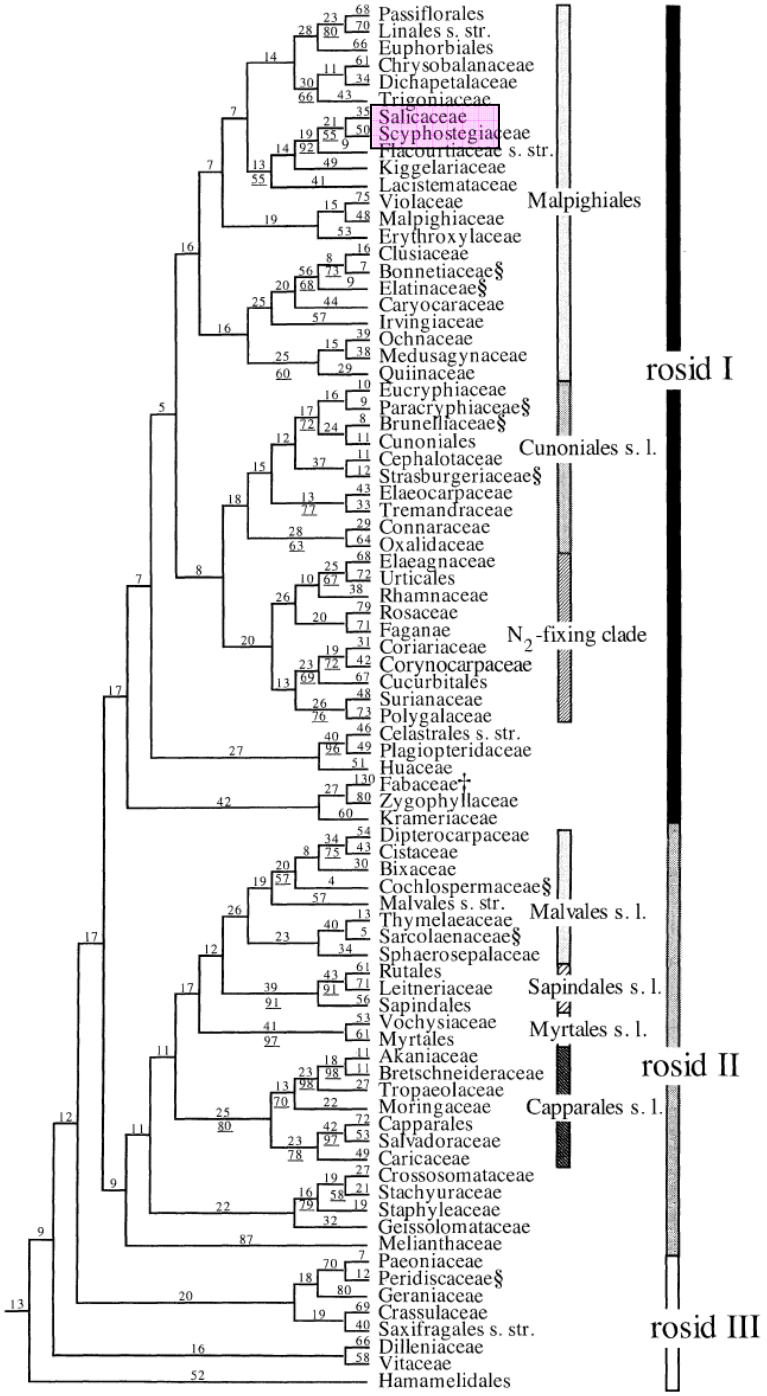

Fig. 54 The single most-parsimonious combined tree found with successive weighting. The tree has 10.271 steps (Fitch length: i.e. equal weights) with CI = 0.16 and RI = 0.38. Numbers avobe the branches are the numbers of estimated changes (ACCTRAN optimization). Underlined numbers below branches are bootstrap values; branches without an underlined number had bootstrap percentages of less than 50%. – A (left). First-branching portion of the tree, arranged with Ceratophyllaceae as the outgroup. Magnoliids form a grade composed of two major subclades (magnolid I and II) with the former sister of the eudicods. Within eudicots, ranunculids and hamamelids form a grade. The caryophyllids are sister to the asterids/rosids (for rosids, see Fig 4B). - B (right). Rosid clade. Note that the glucosinolate and nitrogen-fixing families form clades.

Taxa for *rbc*L sequences were unabailable. + Nitrogen-fixing familiy outside the main nitrogen-fixing clade Fabaceae). From (Nandi et al., 1998) p. 153

4. Genomics of poplars

The bibliography derived from the Web of Science yields 109 published papers on genomics of poplars. Only a few can be mentioned here:

The present state of knowledge has been visualized by (Wullschleger et al., 2002), see fig 1.

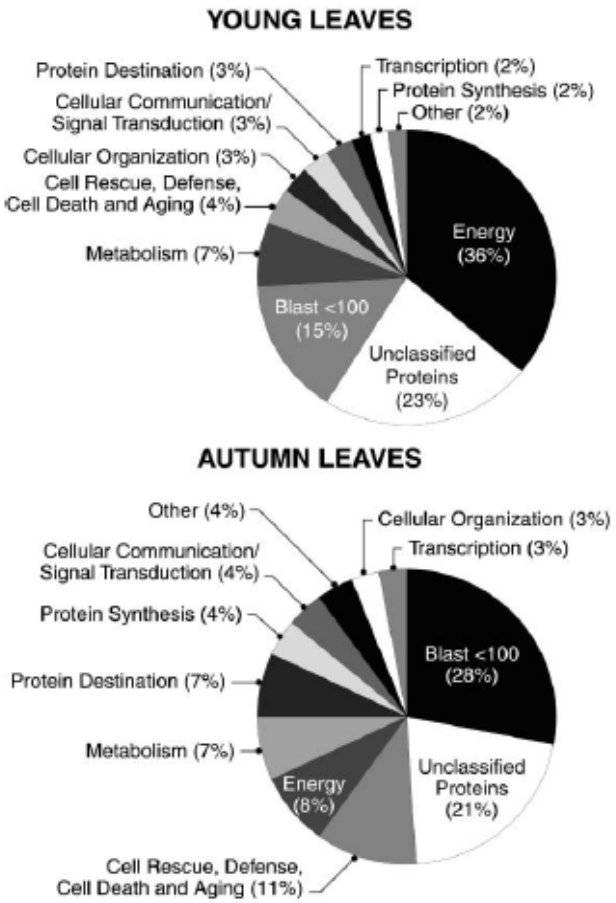

Fig. 55 Functional Distribution of Genes According to a Modified MIPS (Munich Information Center or Protein Sequences) Classification Scheme of 4842 ESTs from Young *Populus* Leaves and 5128 ESTs from Leaves Collected in Autumn. Unclassified proteins show similarity to a gene of unknown function, typically an Arabidopsis open reading frame. Data courtesy of Stefan Jansson, from (Wullschleger et al., 2002).

In a comprehensive study using AFPL markers (Cervera et al., 2005) give the following Dendrogram:

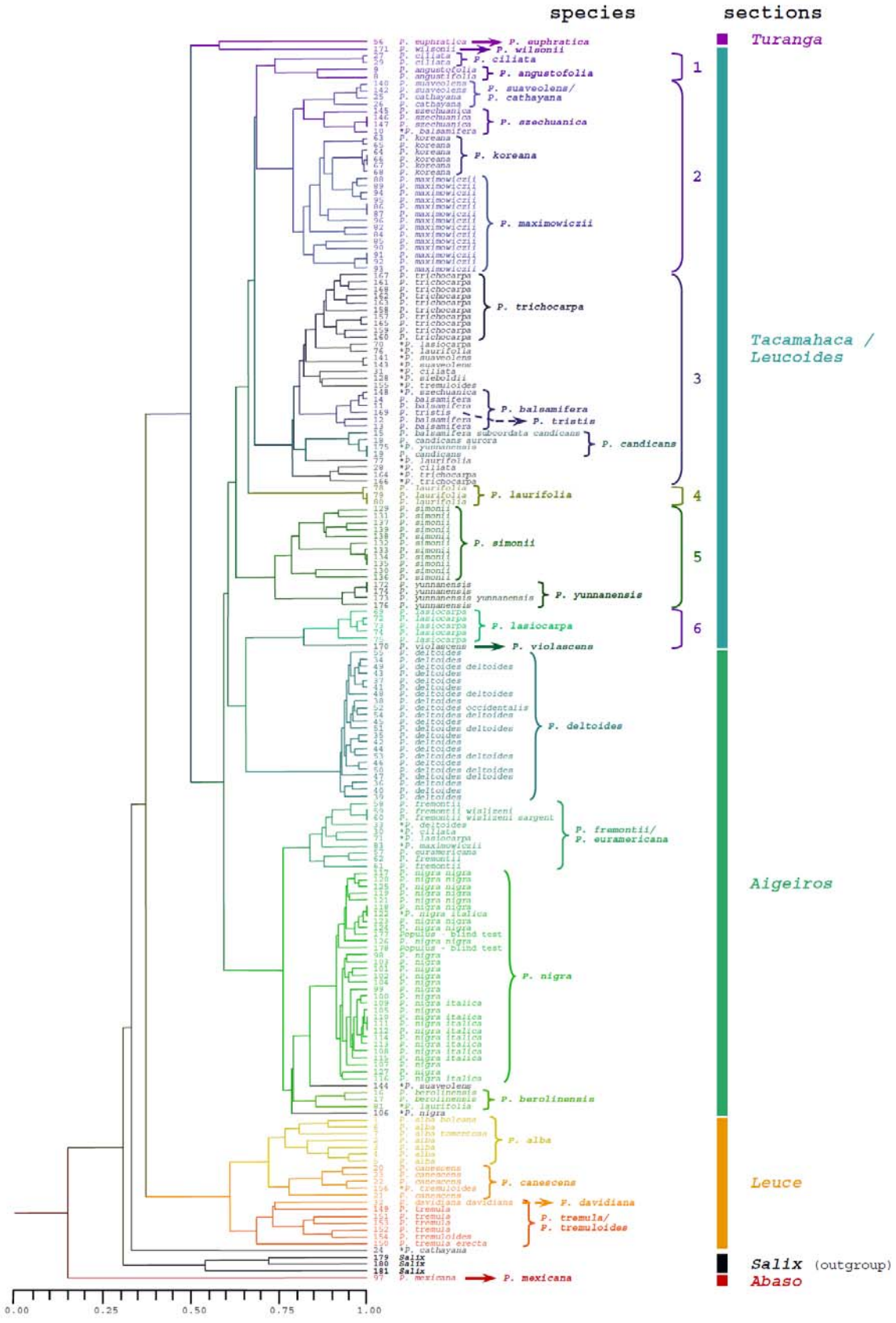
species
sections
Turanga
Tacamahaca / Leucoides
Aigeiros
Leuce
Salix (outgroup)
Abaso
1
2
3
4
5
6
P. euphratica
P. wilsonii
P. ciliata
P. angustifolia
P. suaveolens/ P. cathayana
P. szechuanica
P. koreana
P. maximowiczii
P. trichocarpa
P. balsamifera
P. tristis
P. candicans
P. laurifolia
P. lasiocarpa
P. violascens
P. deltoides
P. fremontii/ P. euramericana
P. nigra
P. berolinensis
P. alba
P. canescens
P. davidiana
P. tremula/ P. tremuloides
P. mexicana
0.00
0.25
0.50
0.75
1.00

Fig. 56 Dendrogram of Populus and Salix accessions, constructed from AFLP fragment similarities (Dice coefficient), with the UPGMA clustering method, and based on AFLP markers resolved by five primer combinations (EcoRI+ATA/MseI+ACAA, EcoRI+ ATA/MseI+ACAC, EcoRI+ATA/MseI+ACAG and EcoRI+ ATA/MseI+ACAT, EcoRI+AAA/MseI+ACAT). Accessions marked with an asterisk are potentially mislabeled species or hybrids (see text and Table 2). Species are marked by brackets and arrows, whereas lines group sections. Fig. 1 from (Cervera et al., 2005)

158 accsessions from 25 species have been used to construct the dendrogram. A dendrogram as well as a single most parsimonious tree, ordered the Populus sections from the oldest Leuce to the latest Aigeiros, a pattern consistent with their known evolutionary relationships. A close relationship between Populus deltoides of the Aigeiros section and species of the Tacamahaca section was observed and, with the exception of Populus wilsonii, between the species of the Leucoides, Tacamahaca, and Aigeiros sections. Populus nigra was clearly separated from its consectional P. deltoides, and should be classified separately from P. deltoides. The AFLP profiles pointed out to the lack of divergence between some species and revealed that some accessions corresponded with interspecific hybrids. This molecular study provides useful information about genetic relationships among several Populus species and, together with morphological descriptions and crossability, it may help review and update systematic classification within the Populus genus.

Although efforts to identify *Populus* as a model tree began long before sequencing a tree genome was a possibility, the choice of poplar was ideal in that the genome size is small, 550 Mbp. This is similar in size to the rice genome, only 4 times larger than the genome of Arabidopsis, yet 40 to 50 times smaller than the genome of pine, (Wullschleger et al., 2002).

Another effort to come to terms with genes and phylogeny concentrating on the comparison with two well known genomes Oryza and Arabidopsis has been recently summarized in (Tuskan et al., 2006) with the following figure:

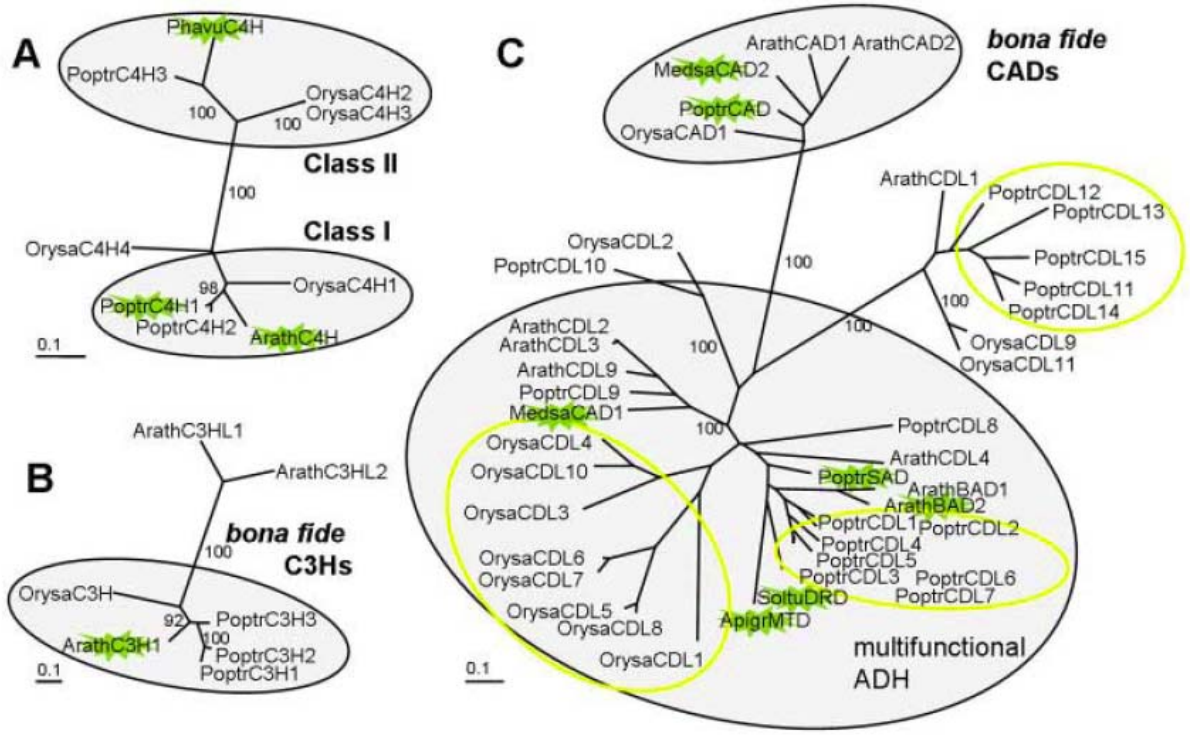

Fig. 57 Phylogenetic analysis of gene families in Populus, Arabidopsis, and Oryza encoding selected lignin biosynthetic and related enzymes. (A) Cinnamate-4-hydroxylase (C4H) gene family. (B) 4-coumaroyl-shikimate/quinate-3-hydroxlase (C3H) gene family. (C) Cinnamyl alcohol dehydrogenase (CAD) and related multifunctional alcohol dehydrogenase gene family. Arabidopsis gene names are the same as those in Ehlting et al. (80). Populus and Oryza gene names were arbitrarily assigned; corresponding gene models are listed in table S13. Genes encoding enzymes for which biochemical data are available are highlighted with a green flash. Yellow circles indicate monospecific clusters of gene family members, from (Tuskan et al., 2006).

According to (Bekkaoui et al., 2003; Brunner et al., 2004; Brunner & Nilsson, 2004; Busov et al., 2003; Cervera et al., 2005; Choi et al., 2006; Christopher et al., 2004; Cole, 2005; Cseke et al., 2005; Djerbi et al., 2005; Johansson et al., 2002; Lescot et al., 2004; Leseberg et al., 2006; Martin & Kohler, 2004; Martin et al., 2004; Moreau et al., 2005; Nicole et al., 2006; Nishiguchi et al., 2002; Park et al., 2004; Ralph et al., 2006; Robinson et al., 2005; Sampedro et al., 2006; Sjodin et al., 2006; Smith & Campbell, 2004; Sterck et al., 2005; Sterky et al., 2004; Stokstad, 2006; Strauss & Martin, 2004; Street et al., 2006; Teeri & Brumer, 2003; Tsai & Hubscher, 2004; Tschaplinski et al., 2006; Tuskan et al., 2006; Tuskan et al., 2004; Yin et al., 2004; Yin et al., 2002; Zalesny & Wiese, 2006; Zhang et al., 2004; Zhang et al., 2005; Zhao et al., 2005a) and many others poplar genomics has made enormous progress from 2002 onwards: Trees present a life form of paramount importance for terrestrial ecosystems and human societies because of their ecological structure and physiological function and provision of energy and industrial materials. The genus populus is the internationally accepted model for molecular tree biology.

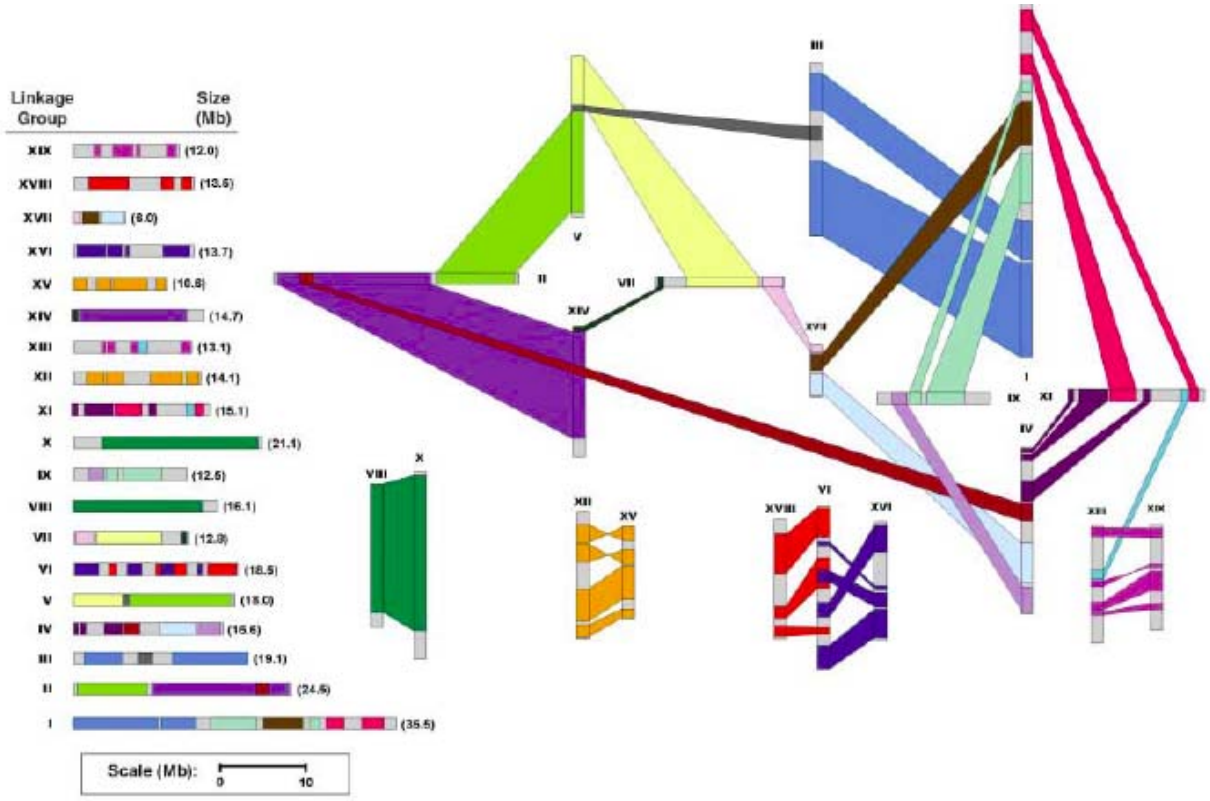

Fig. 58 Chromosome-level reorganization of the most recent genome-wide duplication event in Populus. Common colors refer to homologous genome blocks, presumed to have arisen from the salicoid-specific genome duplication 65 Ma, shared by two chromosomes. Chromosomes are indicated by their linkage group number (I to XIX). The diagram to the left uses the same color coding and further illustrates the chimeric nature of most linkage groups, from (Tuskan et al., 2006)

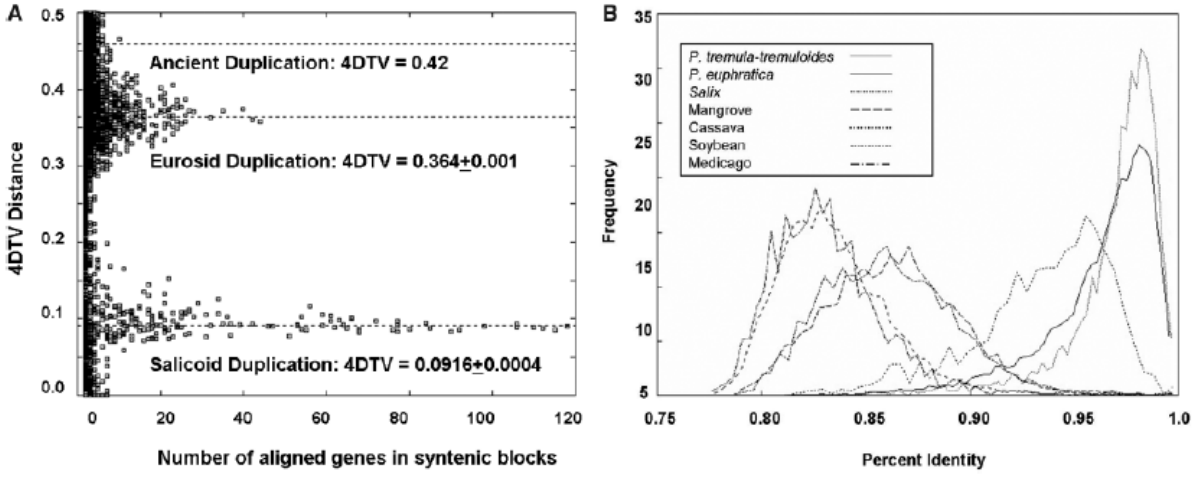

Fig. 59 (A) The 4DTV metrics for paralogous gene pairs in Populus-Populus and Populus-Arabidopsis. Three separate genome-wide duplications events are detectable, with the most recent event contained within the Salicaceae and the middle event apparently shared among the Eurosids. (B) Percent identity distributions for mutual best EST hit to Populus trichocarpa CDS, from (Tuskan et al., 2006).

As a whole, the systematics of Populus will undergo still more revisions, here the 'state of error' given by the OECD document (OECD, 2001b), based on (Eckenwalder, 1996)

Section	Scientific name & synonyms	Common names	Occurrence
Abaso Ecken.			
	P. mexicana Wesmael		Mexico
Turanga Bge.			
	P. euphratica Oliv.	**Euphrates poplar**, bahan	Spain, NE Africa, Asia
	P. ilicifolia (Engler) Rouleau		E. Africa
	P. pruinosa Schrenk		E. Eurasia
Leucoides Spach		**large-leaved poplars**	
	P. lasiocarpa Oliv.	**Chinese necklace poplar**	China
	P. glauca Haines [*P. wilsonii* Schneid.]		China
	P. heterophylla L.	**swamp cottonwood**, swamp poplar,	USA
Tacamahaca Spach		**balsam poplars**	
	P. angustifolia James	**narrowleaf cottonwood**, narrowleaf balsam poplar	southern Sask. And Alberta to southwestern US
	P. balsamifera L.	**balsam poplar**	North America
	P. ciliata Royle		Himalayas
	P. laurifolia Ledeb.	**laurel poplar**	eastern Asia
	P. simonii Carr.	**Simon poplar**	eastern Asia
	P. suaveolens Fish. [*P. cathayana* Rehd. *P. koreana* Rehd *P. maximowiczii* A.Henry]	**doronoki**, Japanese poplar	NE China, Japan
	P. szechuanica Schneid.		E. Eurasia
	P. trichocarpa Torr. & A.Gray	**black cottonwood**, western balsam poplar	western Canada and US
	P. yunnanensis Dode		E. Eurasia
Aigeiros Duby		**Cottonwoods and Black Poplars**	
	P. deltoides Marsh. [*P. sargentii* Dode, *P. wislizenii* Sarg.]	**eastern cottonwood** (ssp. *deltoides*), **plains cottonwood** (ssp. *monilifera*), **Rio Grande cottonwood** (ssp. *wislizenii*	Quebec, Ontario Prairie Provinces to Texas SW USA
	P. fremontii S.Wats.	**Fremont cottonwood**	SW USA
	P. nigra L.	**black poplar**, European black poplar	Europe, western Asia
Populus L. [*Leuce* Duby]		**aspens**	
	P. adenopoda Maxim.		
	P. alba L.	**white poplar**, silver poplar	central and southern Europe to N. Africa, central Asia
	P. gamblei Haines		E. Eurasia
	P. grandidentata Michx.	**largetooth aspen**, bigtooth aspen, aspen, poplar, popple	eastern North America
	P. guzmanantlensis Vasq. & Cue.		Mexico
	P. monticola Brand		Mexico
	P. sieboldii Miq.	**Siebold aspen**, Japanese aspen	Japan
	P. simaroa Rzed.		Mexico
	P. tremula L. [*P. davidiana* (Dode) Schneid.]	**European aspen**, tremble, Zitterpappel	Europe, northern Africa, north-eastern Asia
	P. tremuloides Michx.	**trembling aspen**, quaking aspen	North America

Fig. 60 Suggested classification, nomenclature and occurrence of Populus species (Eckenwalder 1996), and synonyms given by an earlier classification (Zsuffa 1975) in square brackets.

A visualized result of the new relationships is again given by (Cervera et al., 2005).

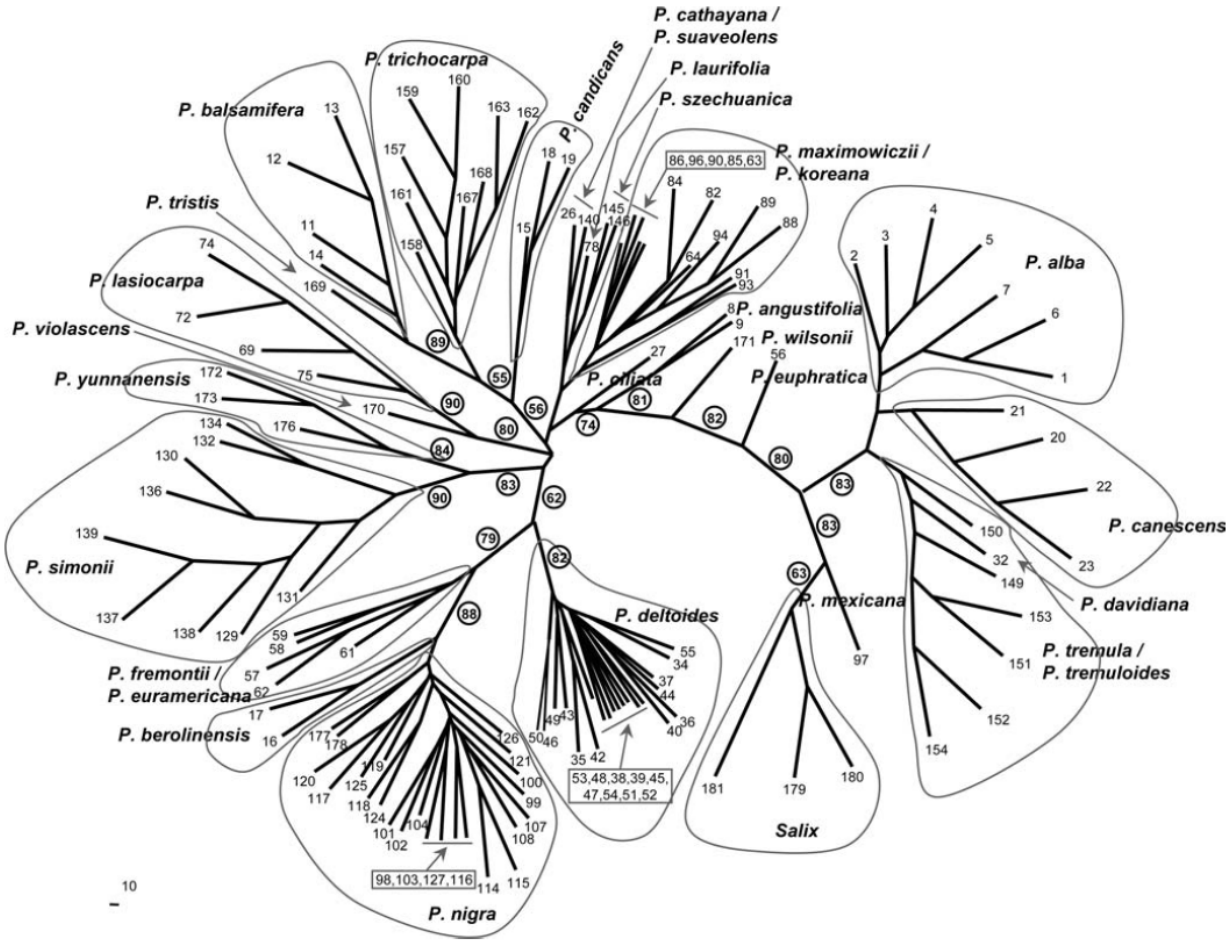

Fig. 61 The single most parsimonious bifurcating unrooted tree, based on the Wagner method, representing the phylogeny of Populus. Plain and circled numbers correspond to accession codes (Table 2) and bootstrap values (only those above 50% are shown for main branches, grouping several species), respectively. Fig 2 from (Cervera et al., 2005).

Among the five original Populus sections, the Leuce and Turanga sections were the most differentiated from the other three sections, based on both phenetic and phylogenetic analyses. The order of the sections in the phylogenetic tree more or less followed their known evolutionary patterns (Eckenwalder 1996), with the oldest Leuce section at one end and the most recent Aigeiros section at the other (Fig. 2). Thus, not only does the AFLP data support previously described evolutionary relationships in the genus Populus (Eckenwalder 1996), but also suggests close genetic relationships between the Aigeiros and Tacamahaca sections. These results are in agreement with the close relationships observed between these sections, which are based on morphology, evolutionary and crossability relationships, and on allozyme and DNA marker analyses (Zsuffa, 1975a) (Eckenwalder, 1984a, b, c, 1996) (Rajora & Zsuffa, 1990) (Barrett et al., 1993) (Rajora & Dancik, 1995).

5. Reproduction biology

The basics of reproduction biology can be checked in the OECD consensus document (OECD, 2001b). Poplars are normally dioecious and obligatory outcrossers; however, the occurrence of monoecious inflorescences and perfect flowers has been reported (Lester, 1963).

Poplars can effectively reproduce by seed production, but also by asexual mechanisms. Tacamahaca and Aigeiros poplars produce large annual seed crops. Those in section Populus produce some seeds each year, but bumper crops occur at intervals of three to five years. Poplars are prolific seed producers. A typical 12 m P. deltoides specimen was estimated to produce almost 28 million seeds in one season, and estimates for P. tremula have ranged as high as 54 million seeds. Poplar seeds are very small and can fly over long distances. Species in section Populus can produce 6000 to 8000 seeds per gram (Schreiner, 1974). Typically, the longevity of poplar seeds under natural conditions is quite short – about two to four weeks. Under controlled low-temperature (-18 to 5° C) and stable moisture content (5 to 8%) conditions, storage time has been extended to 140 days for P. balsamifera (Hellum, 1973), two years for P. tremuloides (Fechner et al., 1981), and five to six years for Aigeiros poplars (Muller & Tessier du Cros, 1982; Tauer, 1979).

Hybridization and seed survival depends largely on ecological conditions:
It should also be noted, that environental conditions can change dramatically the reproduction mode, as shown by many researchers: (Barsoum, 2001; Barsoum et al., 2004; Beaudoin et al., 1992; Fladung et al., 2003; Imbert & Lefevre, 2003; Latva-Karjanmaa et al., 2006; Vanden Broeck et al., 2004). According to (Barsoum et al., 2004) regeneration was overwhelmingly from seed in the first 2–3 years following recruitment, but poor survival rates among sexual recruits saw a shift in the relative abundance of regeneration strategies over time. In relating hydrological data to recruitment, unseasonal flood disturbances had a negative effect on recruitment from seed and a positive effect on vegetative regeneration. Seedlings were associated with fine sediment deposits and were restricted primarily to low elevations on the flood plain, while asexual recruits had a wider spatial distribution.

Except for members of section Populus, all poplars sprout vigorously from the stump and root collar. Coppicing occurs occasionally on young aspen (Zsuffa, 1975a). Reproduction from adventitious shoots on roots (root suckers) is common in many species, although less frequent in those in the Aigeiros and Leucoides sections.

The balance between sexual and asexual propagation in the establishment of natural stands of Populus nigra (Legionnet et al., 1997) assessed genotypes of trees sampled in different natural stands. One site was completely sampled and genotyping proceeded using STS and RAPD markers. Among 118 trees, only four were vegetative copies of other trees of the site. Isozymes were used in four stands, and a method to detect the presence of vegetative copies in a sample is proposed for these markers. No vegetative copies were found in two cases, and a low number of copies was detected in the other

two. Observations are reported for seedlings as well as for different modes of vegetative propagation in natural stands. The authors conclude that sexual and asexual propagation play complementary roles in the dynamics of this species, and that in most cases the adults originate from seedlings.

It is clear from the scheme published by (Zsuffa, 1975b) in fig. 1, that numerous possibilities of hybridization are given, others are prohibited by various instances of biological barriers.

Clonal groups of *P. tremuloides* in eastern North America are very common, but generally less than 0.1 ha in size, while in areas of Utah, groups as large as 80 ha have been observed (Kemperman & Barnes, 1976). In the semi-arid western United States, some argue that widespread seedling establishment has not occurred since the last glaciation, some 10,000 years ago (Einspahr & Winton, 1976; McDonough, 1985). Indeed, some biologists feel that western clones could be as old as 1 million years (Barnes, 1966, 1975). It has been claimed that a single clone, nicknamed "Pando" (Latin for *I spread*), covers 43 hectares, contains more than 47,000 stems and weighs in excess of 6 million kg, making it the largest known organism (Grant et al., 1992; Mitton & Grant, 1996). (This is certainly exceeding the record of a 10’000kg Armadilla fungus reported by (Smith et al., 1992)).

In order to understand the nature of interspecific barriers in Populus, (Villar et al., 1989) have explored pollen/pistil interactions in intra- and interspecific crosses Populus nigra x P. nigra and P. nigra x P. alba. The kinetics of pollen-tube growth demonstrated that P. nigra and P. alba pollen tubes have distinct behavioral patterns inside P. nigra pistils. P. alba pollen tubes exhibit an unique S-shaped growth curve and an arrested growth site near the sylodium. P. nigra pollen tubes exhibit two growth phases, in the stigmatic tissues and in the ovarian cavity respectively. P. nigra and P. alba curves diverge 5 h after controlled pollination and could be related to a change in the physiology of the P. nigra pollen tube, which shifts from an autotrophic to a heterotrophic type of nutrition. Protein analysis of pollinated stigmatic extracts (0,6 and 20 h after pollination) revealed qualitative and quantitative differences that are related to the presence of either P. nigra or P. alba pollen tubes inside the stigmatic tissues. Increasing numbers of protein bands were detectable from 0 to 20 h after pollination only in intraspecific cross. Glycoproteins were detected, and the differences observed were dependent of the cross. -Galactosidase activity was found in pollinated stigmas, but an increase in its activity (one isozyme of pHi 4.2) between 6 h and 20 h after pollination was detected only in the intraspecific cross. This enzyme could play a role in heterotrophic pollen-tube nitrition, and its activity could be the final result of a series of interactions started by the initial pollen-stigma dialog.

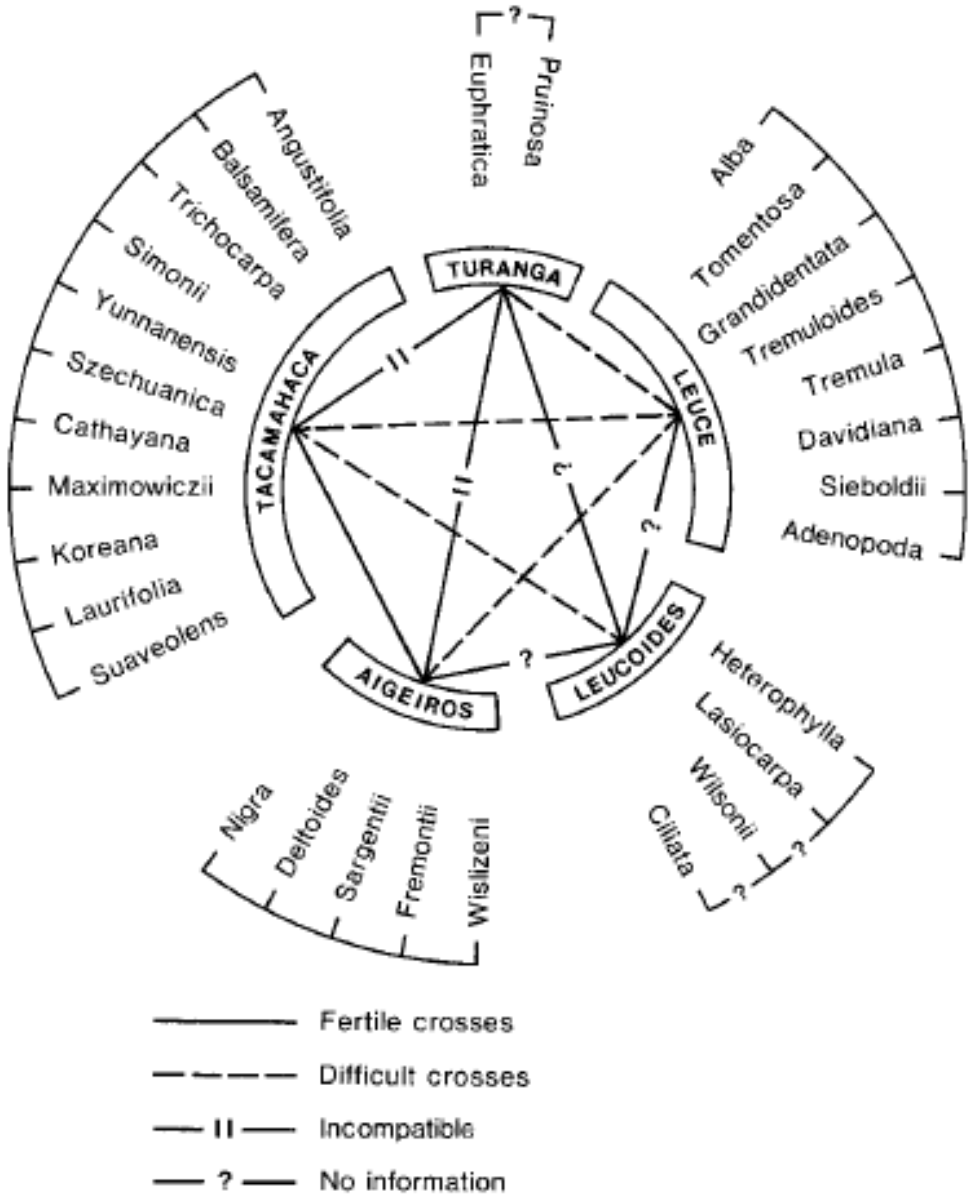

Fig. 62 Crossability of *Populus* species. Extensive crossability studies have been carried out among species in the *Populus (or Leuce)*, *Tacamahaca* and *Aigeiros* sections, while few data are available for those in *Turanga* and *Leucoides* (Zsuffa 1975). Interspecific breeding results are summarised. Fig. taken from (OECD, 2001b)

According to (Gaget et al., 1989) are intersectional crosses between species of sections *Aigeiros* (Populus deltoides, P. x euramericana or P. nigra) and *Populus (Leuce)* (P. alba or P. tremuloides) known to be reciprocally incompatible. The site of pollen tube arrest is on the stigma surface in pollinations between pistils of section *Populus (Leuce)* and pollen of section *Aigeiros*; tubes failed to penetrate the stigma surface. In reciprocal matings, pollen of section *Populus (Leuce)* germinated and tubes penetrated the stigma

and style, where arrest occurred. Rejection may be accompanied by swelling of the tube tips, and callose plug formation. In the cross between P. deltoides and P. alba a callose response was detected in the cell walls of the transmitting tissue, adjacent to the rejected pollen tubes.
These two papers explain the differences in crossing scheme of fig.1.

Hybridization can also be influenced by specific genotypes and specific dynamics of gene flow, which has been shown by an extensive study of (Lexer et al., 2005): Populus alba and Populus tremula hybridize across a large zone of sympatry located in the Danube valley. They genotyped 93 hybrid morphotypes and samples from four parental reference populations from within and outside the zone of sympatry for a genome-wide set of 20 nuclear microsatellites and eight plastid DNA restriction site polymorphisms. Their results indicate that introgression occurs preferentially from P. tremula to P. alba via P. tremula pollen. This unidirectional pattern is facilitated by high levels of pollen vs. seed dispersal in P. tremula and by great ecological opportunity in the lowland floodplain forest in proximity to P. alba seed parents, which maintains gene flow in the direction of P. alba despite smaller effective population sizes in this species. Results indicate that hybrid zones will be valuable tools for studying the genetic architecture of the barrier to gene flow between these two ecologically divergent Populus species.

According to (Hoenicka & Fladung, 2006) we have to face, besides a given set of natural species, from region to region a different set of already existing hybrids in nature, due to various reasons from forestry, horticulture, landscape architect activities etc:

Parentage	Hybrid designation [Synonym] (Common name)
P. alba × *P. grandidentata*	*P.* × *roulwauiana* Boivin
P. alba × *P. Adenopoda*	*P.* × *tomentosa* Carr. (Chinese white poplar)
P. alba × *P. Tremula*	*P.* × *canescens* Ait. Sm. (grey poplar)
P. alba × *P. Tremuloides*	*P.* × *heimburgeri* Boivin
P. angustifolia × *P. deltoides*	*P.* × *acuminata* Rydb.[syn. P. × andrewsii Sarg.] (Lanceleaf cottonwood)
P. angustifolia × *P. balsamifera*	*P.* × *brayshawii* Boivin (Brayshaw's poplar)
P. angustifolia × *P. tremuloides*	*P.* × *sennii* Boivin
P. balsamifera × *P. deltoides*	*P.* × *jackii* Sarg. (Jack's poplar)
P. balsamifera × *P. tremuloides*	*P.* × *dutillyi* Lepage
P. deltoides × *P. Nigra*	*P.* × *canadensis* Moench cv. Eugenei [syn. *P.* × *euramericana* Dode Guinier] (Carolina poplar, Canada poplar, Euramerican poplars)
P. deltoides × *P. tremuloides*	*P.* × *bernardii* Boivin (Bernard poplars)
P. deltoides × *P. Trichocarpa*	*P.* × *generosa* Henry [syn. *P.* × *interamericana* Brockh.] (Interamerican poplars)
P. fremontii × *P. trichocarpa*	*P.* × *parryi* Sarg. (Parry cottonwood)
P. grandidentata × *P. Tremuloides*	*P.* × *smithii* Boivin
P. laurifolia × *P. Nigra*	*P.* × *berolinensis* Dippel [syn. *P.* × *rasumowskyana* Schr. and *P.* × *petrowskyana* Schr.] (Berlin poplars, Russian poplars)
P. deltoides × *P. balsamifera* × *P. angustifolia* (natural trihybrid)	Unnamed

Fig. 63 Natural and introduced *Populus* hybrids in the environment, modified from (OECD, 2001b), from (Hoenicka & Fladung, 2006)

A well known example is the frequent hybridization of Populus alba and canescens, sometimes with mixed in Populus balsamifera, producing still unnamed triple hybrids, as documented in fig. 2 above.

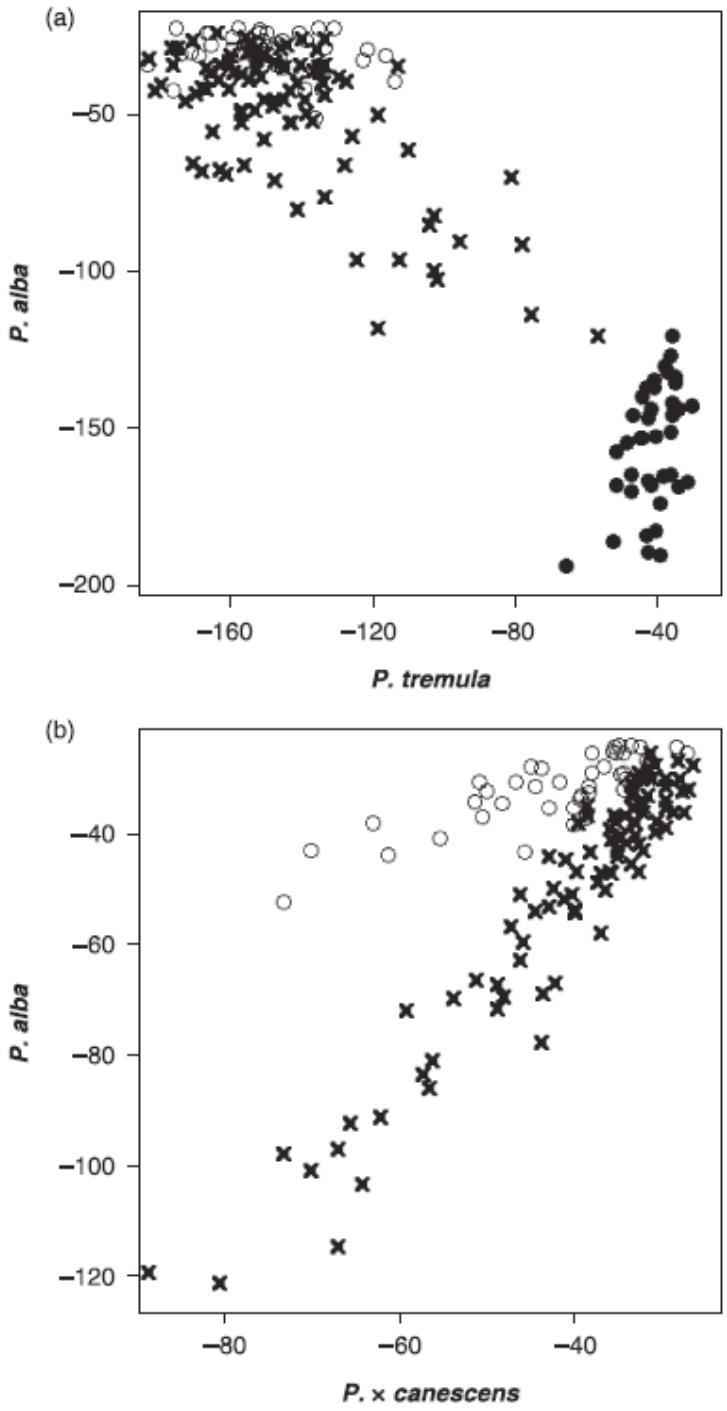

Fig. 64 Log-likelihoods of microsatellite-based genotype assignments to *Populus alba* vs. *Populus tremula* (Fig. 2a) or *P. alba* vs. *P.* × *canescens* hybrids (Fig. 2b). Taxon designations used to perform the analyses were based on leaf morphology and were tested by Bayesian admixture analysis prior to the assignment tests. Open circles, *P. alba*; filled circles, *P. tremula*; crosses, *P.* × *canescens* hybrids, from (Lexer et al., 2005)

6. Risk assessment of transgenic poplars:

6.1. Long term aspects of tree biology

In contrast to other crops, risk assessment in poplar is faced with a special long term problem: Generation span is much longer, and here even more than with normal species we have to take into account long term problems of environmental behavour of alien species, which has been studied extensively by (Kowarik, 1992a, 1995, 1999, 2003a, b, 2005).

Tree species	Time-lag (years)
Prunus persica	415
Juglans regia	374
Thuja occidentalis	324
Fraxinus ornus	246
Corylus colurna	222
Laburnum anagyroides	198
Acer negundo	183
Celtis occidentalis	172
Robinia pseudoacacia	152
Populus × canadiensis	165
Aesculus hippocastanum	124
Ailanthus altissima	122
Pinus strobus	117
Quercus rubra	114
Sorbus intermedia	112
Pseudotsuga menziesii	112
Prunus mahaleb	54
Prunus serotina	29

Fig. 65 Time-lags between the first introduction of non-native trees to Brandenburg/Germany and the beginning of an invasion process, after (Kowarik, 1992b, 2003a) from (Hoenicka & Fladung, 2006).

Trees species (wild/hybrid)	Conflicts with			Initial introduction		Pathways of secondary releases							
	Agriculture	Nature conservation	Silviculture	Deliberate	New taxa	Garden ornamental	Plant for hedges/ shelterbelts	Silvicultural crops	Soil improvement	Erosion control	Beekeeper's plant	Game shelter/forage
Acer negundo		□		□		■	■					
Pinus nigra		□		□		■		■				
Pinus strobus		□		□		■		■				
Populus × euroamericana		□			□	■	■	■				
Prunus serotina		□	□	□		■	■	◊	◊			
Quercus rubra		□		□		■		■	■	■	■	■
Robinia pseudoacacia	□	□		□		■	■	■				
Pseudotsuga menziesii		□		□		■		■				

Additional information: The relevance of pathways has been estimated for Germany as (■) recently relevant, (◊) relevant only before 1950

Fig. 66 Problematic exotic tree species (wild or hybrid ones) subject to control in Germany, after (Kowarik, 2003a), adapted, from (Hoenicka & Fladung, 2006)

Comments: There is only one Populus taxon under special surveillance in Germany, for reasons it shows invasive character: P. x euroamericana (the hybrid between Populus deltoides and P. nigra).

6.2. Transgenic poplars in literature, a mini-review

A bibliographic search on the Web of Science reveals nearly 100 published papers on transgenic poplars:
http://www.botanischergarten.ch/EPOBIO/Bibliography-WOS-transgenic-poplar-20061229.pdf

Overall, the number of tree species for which in vitro propagation and transformation protocols exist is growing, reviewed in (Tzfira et al., 1998), as well as the number of genetically modified trees being tested in field trials (approx. 140 field-trials with 17 species) (McLean & Charest, 2000; Mullin & Bertrand, 1998). Beside the pioneer genus *Populus* tree biotechnology concentrates on transformation of conifers, where only a few stable transgenic lines exist, and economically important species such as *Eucalyptus*, grape, and apple.
As in crop plant biotechnology, the first traits expressed in trees and tested in field release experiments were insect, herbicide, and disease resistance (Jouanin et al., 1998; Robinson, 1999; Séguin et al., 1998). Genes coding for *Bacillus thuringiensis* toxins were introduced into
several tree species including poplar, walnut, white spruce, and larch. Another strategy for engineering insect resistance was employed by overexpressing proteinase inhibitors in poplar, resulting in toxicity of such plants for *Chrysomela tremulae(Genissel et al., 2003; Leple et al., 1995)* or *Plagiodera versicolora* (Klopfenstein et al., 1997; Klopfenstein et al., 1991). Transgenic aspens and poplars were manipulated for resistance against the herbicides glyphosate (Donahue & Michler, 1993), chlorsulfuron (Brasileiro et al. 1992), and phosphinotricine

(DeBlock 1990; Jouanin et al. 1993). Papaya overexpressing the ring-spot virus coat protein and thus less susceptible to infection by this virus (Gonsalves 1998) was the first commercial transgenic tree (McLean and Charest 2000; Chiang et al. 2001).

In a review on transgenic trees as tools in tree and plant physiology (Herschbach & Kopriva, 2002) the following summary scheme is helpful

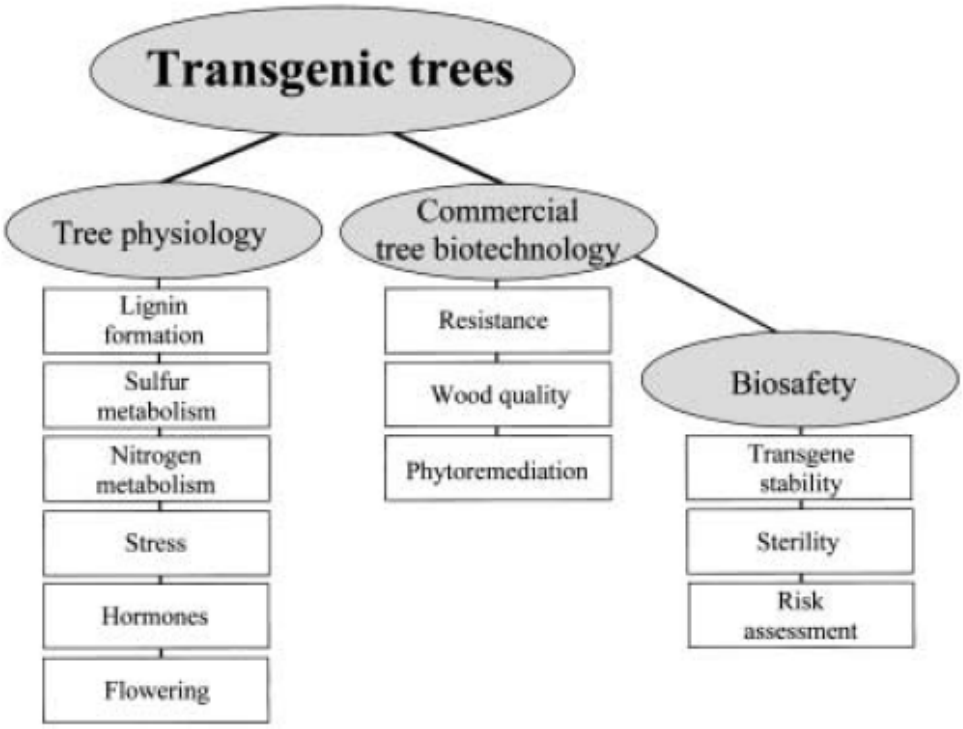

Fig. 67 Transgenic trees in tree physiology and biotechnology from (Herschbach & Kopriva, 2002)

Four tables below give insight in the range of Transgenity in trees, all from (Herschbach & Kopriva, 2002)

Table 1 List of transgenic trees overexpressing genes involved in sulfur or nitrogen metabolism

Tree species	Gene overexpressed	Origin of the coding sequence	Compartment	Promoter	Line coding	Literature
Populus tremula × *P. alba*	γ-Glutamylcysteine synthetase	*Escherichia coli*	Cytosol	CaMV 35S	gsh28 ggs	Noctor et al. (1996); Arisi et al. (1997)
Populus tremula × *P. alba*	γ-Glutamylcysteine synthetase	*Escherichia coli*	Chloroplast	CaMV 35S	Lggs	Noctor et al. (1998a)
Populus tremula × *P. alba*	Glutathione synthetase	*Escherichia coli*	Cytosol	CaMV 35S	gsh	Foyer et al. (1995); Arisi et al. (1997)
Populus tremula × *P. alba*	Glutathione synthetase	*Escherichia coli*	Chloroplast	CaMV 35S	Lgsh	Noctor et al. (1998a)
Populus tremula × *P. alba*	Glutathione reductase	*Escherichia coli*	Cytosol	CaMV 35S	35 gor	Foyer et al. (1995)
Populus tremula × *P. alba*	Glutathione reductase	*Escherichia coli*	Chloroplast	CaMV 35S	70 L gor	Foyer et al. (1995)
Populus tremula × *P. alba*	Glutamine synthetase	*Pinus pinaster*	Cytosol	CaMV 35S		Gallardo et al. (1999)

Table 2 List of transgenic trees manipulated in genes involved in stress defense

Tree species	Gene manipulated	Origin of the coding sequence	Compartment	Promoter	Orientation	Literature
Liriodendron tulpifera	Mercuric reductase (merA)	*Escherichia coli*	Cytosol	CaMV 35S	Sense	Rugh et al. (1998)
Populus tremula × *P. alba*	Fe superoxide dismutase (FeSOD)	*Arabidopsis thaliana*	Chloroplast	CaMV 35S	Sense	Arisi et al. (1998)
Populus tremula × *P. alba*	Chalcone synthase (chs)	*Petunia*	Cytosol	CaMV 35S	Sense	Nicolescu et al. (1996)
Juglans nigra × *J. regia*	Chalcone synthase (chs)	Walnut		CaMV 35S	Antisense	Euch et al. (1998)

Table 3 List of transgenic trees manipulated in hormone contents

Tree species	Gene overexpressed	Origin of the coding sequence	Compartment	Promoter	Orientation	Literature
Populus tremula × *P tremuloides*	Trp-2-mono-oxygenase (*iaaM*), indole-3-acetamine hydrolase (*iaaH*), *iaaM* + *iaaH*	*Agrobacterium tumefaciens*	Cytosol	Mannopine synthase (*iaaM*) CaMV 35S (*iaaH*)	Sense	Tuominen et al. (1995)
Populus tremula × *P tremuloides* expressing *iaaH*	Trp-2-mono-oxygenase (*iaaM*)	*Agrobacterium tumefaciens*	Cytosol	RolC	Sense	Tuominen et al. (2000)
Populus tremula × *P tremuloides*	GA-20-oxidase	*Arabidopsis thaliana*	Cytosol	CaMV 35S	Sense	Eriksson et al. (2000)
Populus tremula × *P tremuloides*	Phytochrome A (phyA)	Oat	Cytosol	CaMV 35S	Sense	Olsen et al. (1997)
Malus domestica	Phytochrome B (phyB)	*Arabidopsis*	Cytosol	CaMV 35S	Sense	Holefors et al. (2000)
Populus nigra	Homeobox *OSH1*	Rice	Cytosol	CaMV 35S	Sense	Mohri et al. (1999)
Populus tremula × *P. alba*	Isopentenyl transferase (ipt)	*Agrobacterium tumefaciens*	Cytosol	Ipt	Sense	Von Schwartzenberg et al. (1994)
Populus sieboldii × *P. grandidentata*	Isopentenyl transferase (ipt)	*Agrobacterium tumefaciens*	Cytosol	CaMV 35S	Sense	Ebinuma et al. (1997)
Populus tremula × *P tremuloides*	*rolC*	*Agrobacterium rhizogenes*	Cytosol	CaMV 35S	Sense	Fladung et al. (1996); Nilsson et al. (1996a)
Populus tremula × *P tremuloides*	*rolC*	*Agrobacterium rhizogenes*	Cytosol	rbcS	Sense	Fladung et al. (1997)
Populus tremula	*rolB* + *rolC*	*Agrobacterium rhizogenes*	Cytosol	rolC	Sense	Tzfira et al. (1998b, 1999)
Malus domestica	*rolB*	*Agrobacterium rhizogenes*	Cytosol	rolB	Sense	Welander et al. (1998); Zhu et al. (2001)
Malus domestica	*rolA*	*Agrobacterium rhizogenes*	Cytosol	rolA	Sense	Holefors et al. (1998)
Poncirus trifoliata	*rolC*	*Agrobacterium rhizogenes*	Cytosol	CaMV 35S	Sense	Kaneyoshi and Kobayashi (1999)
Actinidia deliciosa	*rolA, rolB* +*rolC*	*Agrobacterium rhizogenes*	Cytosol	rolA, rolB + rolC	Sense	Rugini et al. (1991)
Pyrus communis	*rolC*	*Agrobacterium rhizogenes*	Cytosol	rolC	Sense	Bell et al. (1999)

Table 4 List of transgenic trees manipulated in flowering

Tree species	Gene manipulated	Origin of the coding sequence	Compartment	Promoter	Orientation	Literature
Populus tremula × *P. tremuloides*	*LEAFY*	*Arabidopsis thaliana*	Cytosol	CaMV 35S	Sense	Weigel and Nilsson (1995)
Populus tremula × *P. alba*, *P. tremula* × *P. tremuloides*, *P. trichocarpa* × *P. deltoides*	*LEAFY*	*Arabidopsis thaliana*	Cytosol	CaMV 35S	Sense	Rottmann et al. (2000)
Populus tremula × *P. alba*, *P. tremula* × *P. tremuloides*, *P. trichocarpa* × *P. deltoides*	*PTLF* (*LEAFY* homolog)	*Populus trichocarpa*	Cytosol	CaMV 35S	Sense, antisense	Rottmann et al. (2000)
Citrus sinensis × *Poncirus trifoliata*	*LEAFY*	*Arabidopsis thaliana*	Cytosol	CaMV 35S	Sense	Peña et al. (2001)
Citrus sinensis × *Poncirus trifoliata*	*APETALA1*	*Arabidopsis thaliana*	Cytosol	CaMV 35S	Sense	Peña et al. (2001)

Fig. 68 Tables 1-4: list of transgenic trees for various tansgene traits

6.3. Risk assessment: some characteristics for transgenic trees and poplar in particular

Pollen mediated gene flow is important, but vegetative propagation has to be considered as well, as already stated by the OECD consensus paper (OECD, 2001b): Seed production in poplars starts usually between the age of 10 to 15 years (Schreiner, 1974). In the case of Populus deltoides flowers may appear as early as in age four years (Farmer & Pitcher, 1981).
In their field studies assessing the gene flow risk of transgenic poplars (Fladung et al., 2003) concluded that more than half of the roots suckers investigated showed the presence of the rbcS-rolC gene construct. They concluded that in addition to the widely accepted generative propagation, vegetative dispersal capacity of transgenic perennial plants is also important and must be included in risk assessment studies.

In a very instructive paper, (Strauss et al., 2004) summarize important points to be considered in the biosafety research and communication for transgenic poplars:
Based on extensive experience with transgenic poplars in laboratory and field environments, we have found that transformation is an extremely useful tool for research in biotechnology and functional genomics. The key lessons related to biosafety from their experience are:

Stable gene expression is the rule in vegetatively propagated transgenic poplars:
Instability of gene expression has been reported in studies of transgenic poplar that employ transgenes whose variation in expression is dramatically amplified by its effects on plant development (e.g. *rol*B; (Kumar & Fladung, 2001). However, there is as yet no evidence that expression of transgenes under vegetative propagation is more variable than expression of most endogenes. Normal variation in expression might be dramatically amplified by linkage to potent growth regulatory protein.
(Strauss et al., 2004) have studied stability of gene expression extensively in transgenic poplars using easily visualized marker genes (β-glucuronidase (GUS), herbicide tolerance, and/or insect resistance) and, in some cases, native poplar genes (Rottmann et al., 2000). Based on more than 1000 independent events produced by the Strauss research group containing one or more transgenes have yet to produce a single case of obvious post-regeneration gene silencing despite many rounds of vegetative propagation, annual cycles of growth, and diverse environments to which they have been subjected (Meilan et al., 2002a; Meilan et al., 2004a). However, as long as the major effective transgenes have not been determined, stability data remain generally insufficient. Some authors have data demonstrating higher expression instability employing transgenes whose variation is dramatically amplified by its effects on plant development, e.g. *rol*B in (Kumar & Fladung, 2001).

Somaclonal variation is modest and manageable:
Only ca. 0.06 % morphological abnormalities, not linked to the transgenes were observed by (Meilan et al., 2002a; Meilan et al., 2004a; Meilan et al., 2002b; Meilan et al., 2004b) reported in (Strauss et al., 2004), but only after trees had gone through dormancy. Other laboratories appear to have observed higher levels of somaclonal

variation than we have (Fladung & Kumar, 2002; Wang et al., 1996). However, none appear to be so high as to pose a significant constraint on commercial programmes.

Gene flow is complex and needs careful consideration:
Already the OECD study claimed a rather high gene flow rate (OECD, 2001b).
Poplars and trees present great advantages and difficulties when considering transgene dispersal into the environment, either via seeds, pollen or asexual propagation. The key advantage is that, with the limited planting of transgenics contemplated for the foreseeable future, gene flow from wild and planted non-transgenic trees are certain to astronomically swamp levels of gene flow from plantations, especially if low fertility hybrids or incompletely sterile transgenics are employed, as discussed in (DiFazio et al., 2004). However, poplars also show the ability for vegetative propagation. In nature, this is likely to mainly affect local spread; however, it also facilitates legal and illegal movement by humans. Poplars also have a high propensity for long-distance pollen movement by wind, and seed can be spread via wind and water. This means that unless there is complete sterility, some level of very long-distance migration is likely – this can raise social or legal concerns even if near-astronomical dilution renders it of extremely low biological consequence. Spread is also constrained by a number of very complex factors, including habitat suitability (poplars generally require highly disturbed, moist sites that are free of plant competition), frequency of disturbance, rotation age relative to onset of flowering, and fitness benefit/detriment from transgenes. (DiFazio et al., 2004) have developed a simulation model called STEVE (DiFazio, 2002) which they used to estimate levels of gene flow from transgenic poplar plantations over a 50–100-year period. However, far less sophisticated methods could be used to develop order-of-magnitude gene flow estimates based on relative areas, ages and proximities of transgenic to non-transgenic tree populations.
The STEVE model responded as expected to changes in fertility, competitiveness, dispersal, disturbance, and management activities, though some unexpected behaviors emerged as well. Enhanced competitive ability led to a striking increase in transgene flow, especially under enhanced disturbance. Extrapolation of trends suggests transgenes would introgress extensively in native tree populations, with the rate depending on disturbance and the magnitude of the competitive advantage. However, it is unlikely that oligogenic changes produced with current technology could lead to such a strong, uniform, and long-term fitness benefit in the wild. Further research in this area is highly desirable.

The issue of enhanced competitiveness of transgenics may be largely obviated by the use of transgenic trees with greatly reduced fertility. Simulations showed that fertility reductions of 90% or more greatly slowed or prevented the spread of transgenics. However, the structure of the STEVE model causes an overestimation of gene flow at low fertility levels. Therefore, risk assessments for low-fertility transgenics will be conservative in this respect with the current version of the model.

Sterility systems can be developed via diverse means:
For transgenes whose uncontrolled dispersal could create management problems, violate management conditions mandated by certification systems (Strauss et al., 2001a) or pose ecological uncertainties that are hard to quantify, gene containment via

engineered sterility has long been an important goal (Strauss et al., 1995). This confinement is likely to be essential for genes such as those used for pest/herbicide resistance and bioremediation, especially when the genes employed are of an exotic origin and not required to meet a pressing environmental or social problem. However, social considerations might dictate that containment be required for all transgenes, including those that would not increase fitness, such as wood-modification genes (Doering, 2004).

For transgenes that could sustainably promote fitness in interfertile wild-tree populations, long-term demonstrations of complete, stable sterility (if this is achievable) might be required before any commercial use is allowable or advisable. Alternatively, such transgenics might be banned entirely, either regionally, nationally or globally. We do not believe that single Bacillus thuringiensis toxin genes should necessarily require absolute confinement; insects are expected to readily evolve to overcome their effects, greatly diminishing their selective value and spread, over modest evolutionary time frames (Strauss et al., 2001b). The final conclusion about deployment of such genes will rest on the impact of the target pest, the environmental risks and costs of alternative control measures, including chemicals and irreversibly released biocontrol organisms, and the costs of doing nothing at all. For the foreseeable future, sterility systems are likely to need redundancy to ensure a high level of confinement, thus research on diverse mechanisms is desirable.

Among other experiments, the team of Strauss are studying several ways to use these genes to induce sterility, including their suppression (singly and together via RNA interference), directing a cell toxin to destroy floral tissues (ablation), and production of mutant protein forms that interfere with normal protein function (dominant negative mutants, DNMs). These studies are in various stages, ranging from identification of novel DNMs in Arabidopsis to evaluation of transgenic trees in the field. The long delay until flowering in poplars (3–6 years normally) and the difficulties of measuring sterility in large flowering trees, pose considerable logistical hurdles to the speed and accuracy of this kind of research.

We should remain realistic and also acknowledge that there is still a long way to a reproductive containment system based on reproduction biology with poplar: Efforts to induce the production of precocious, fertile flowers and seed via transgenes have thus far proven unsuccessful (Rottmann et al., 2000) Table 1:

Gene (plasmid construct)	Poplar genotype*	No. PCR-verified events	References	Result
35S-PTLF (p104S)	353	16	Rottmann *et al.* (2000)	Very rare early floral onset, some morphological disturbance
35S-LFY (pDW151)	353 717 184–402 189–434 24–305 17–50	20 13 7 2 2 5	Weigel and Nilsson (1995), Rottmann *et al.* (2000), Skinner *et al.* (2003)	Early flowering but genotype specific, single flowers (not catkins), most effective for males, flowers infertile, highly branched/dwarf vegetative form
35S-AP1 (pAM563)	353 717 184–402	3 10 3	Mandel and Yanofsky (1995)	No flowers, normal vegetative form
35S-OsMADS1 (pGA1209)	353	6	Chung *et al.* (1994)	No flowers, normal
35S-CONSTANS (pART27/CO)	353 717 184–402 17–50 19–53	10 10 10 1 2	Putterill *et al.* (1995)	No flowers, normal vegetative form
35S-AGL20 (pSK231)	353 717 184–402 17–50	10 10 10 1	Rounsley *et al.* (1995)	No flowers, normal vegetative form

Transgenic poplars were produced in 1994–96, planted in pots in the autumn of 1996 and 1997, and monitored in the glasshouse for 3–7 years.
PCR (polymerase chain reaction) was used to verify presence of the target floral transgene in transformed plants.
*The parental genotypes were: INRA 353–53 (male (M), *Populus tremula* × *P. tremuloides*); INRA 717–1B4 (female (F), *P. tremula* × *P. alba*); 184–402 (F, *P. trichocarpa* × *P. deltoides* (TD)); 189–434 (unknown, TD); 24–305 (M, TD); 19–53 (F, TD) and 17–50 (F, TD). All TD hybrids listed are triploids.

Fig. 69 Flowering genes tested in regenerated transgenic poplar whose over-expression had accelerated onset of flowering in *Arabidopsis* or other annual plant species, from (Strauss et al., 2004)

Domestication of transgenes can provide new avenues to promote biosafety.
In short, transformation in poplar is extremely reliable and there are diverse and promising means for improving biosafety, but considerable time, institutional commitments and public–private partnerships are required to deliver them to society. There are other ways and means to mitigate gene flow, but unfortunately, the long breeding cycles make things more difficult. Promising pathways of mitigation have been proposed by (Al-Ahmad et al., 2006a; Al-Ahmad et al., 2004, 2006b; Al-Ahmad & Gressel, 2005, 2006; Gressel & Al-Ahmad, 2005): They developed tandem constructs, which would avoid or at least reduce considerably the spread of transgenes, the concept has been proven to produce stable gene constructs and sustainable mitigation:

Transgenic mitigation (TM), where a desired primary gene is tandemly coupled with mitigating genes that are positive or neutral to the crop but deleterious to hybrids and their progeny. This was tested experimentally by the team of Gressel from Rehovot in Israel as a mechanism to mitigate transgene introgression. Dwarfism, which typically increases crop yield while decreasing the ability to compete, was used as a mitigator. A construct of a dominant ahasR (acetohydroxy acid synthase) gene conferring herbicide resistance in tandem with the semidominant mitigator dwarfing Δ gai (gibberellic acid-insensitive) gene was transformed into tobacco (Nicotiana tabacchum). The highest reproductive TM fitness relative to the wild type was 17%. The results demonstrate the suppression of crop–weed hybrids when competing with wild type weeds, or such crops as volunteer weeds, in seasons when the selector (herbicide) is not 273 used. The linked unfitness would be continuously manifested in future generations, keeping the transgene at a low frequency.

Summary remarks to existing papers on biosafety of transgenic poplars
A search in the literature reveals only a very few papers which deal with real time and real field data on trials done for the clarification of biosafety issues with transgenic poplars: (Abrahamson et al., 1990; Cassens & Eslyn, 1983; Jing et al., 2004; Meilan et al., 2002a; Strauss et al., 2004). They yield data without exception that the situation can be managed and kept under control. The exception is (Hoenicka & Fladung, 2006) which give a lot of citations about *potential* detrimental effects of gene flow. In particular also on horizontal gene flow – but a thorough analysis of their cited papers on HGT reveals that all paper from the school of Kornelia Smalla are not given (Gebhard & Smalla, 1999; Lynch et al., 2004; Nielsen et al., 1998; Nielsen et al., 1997; Nielsen et al., 2000a; Nielsen et al., 2000b, 2001; Smalla & Sobecky, 2002). It is exactly those papers which all come to the conclusion that HGT from higher plants to microorganisms is not an issue in field releases of transgenic plants. Nevertheless: the paper of (Hoenicka & Fladung, 2006) is still an excellent review on all *potential* issues in the release of transgenic organisms into nature, but it lacks in many cases experimental data. And it should be stressed that we need a *risk-benefit* analysis, not a onesided analysis of *potential* risks.

6.4. Biosafety evaluation of the field release of transgenic poplars according to the Dutch-Swiss-Irish method

The Dutch-Swiss-Irish method of classification of risks is based on several previous papers from Holland and Switzerland: Based on the two first publications (Frietema, 1994, 1996) a Swiss research group developed the evaluation system further on: (Ammann & Jacot, 2003; Ammann et al., 1999; Ammann et al., 1996, 2000, 2003). A latest improvement has been achieved by (Flannery et al., 2005), presenting an extended evaluation scheme, including now also seed propagation. In this study, we extend it again with vegetative propagation potential (tillering, included in the evaluation lines for the seed propagation), in order to adapt the system to the reproduction biology characteristics of poplars. Two new strands are also added:
CGC: Consequences of Gene Flow and
CMG: Mitigation Gene Flow crop to crop, crop to wild relative and crop to feral

In a summary, (Flannery et al., 2005) proposed an extended evaluation scheme:

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 in Ireland?	0/1
CPW2	Is there a probability that the crop will flower and produce viable pollen during its cultivation?	0/1
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)?	1/2/3/4/5/6
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)?	0/1/2
CPW5	If fertilization is achieved by the deposited pollen, will a viable F_1 hybrid individual establish itself?	0/1

Strand	Question	Score
CPC	**Propensity for successful pollen-mediated gene flow between the crop and related commercial varieties**	
CPC1	Is there a probability that the crop will flower and produce viable pollen during its cultivation?	0/1
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)?	1/2/3/4/5/6
CPC3	If flowering does occur is the receptive crop rated as an obligate inbreeder (0), a partial inbreeder/ outbreeder (1) or an obligate outbreeder (2)?	0/1/2
CPC4	If fertilization is achieved by the deposited pollen, will a viable F_1 individual establish itself from the hybrid seed in the absence of mechanical/chemical control?	0/1

Strand	Question	Score
CSV	**Propensity for successful seed-mediated* gene flow from commercial crop to volunteer**	
CSV1	Does the crop produce seed during its cultivation?	0/1
CSV2	Post-harvest, will the seed survive and germinate within the confines of a managed field?	0/1
CSV3	Will the volunteer develop into a viable individual?	0/1

Strand	Question	Score
CSF	**Propensity for successful seed-mediated* gene flow from commercial crop to feral**	
CSF1	Does the crop produce seed during its cultivation?	0/1
CSF2	Following transfer from the site of cultivation will wayward seed survive and germinate?	0/1
CSF3	Will the resulting individuals establish into a viable feral population?	0/1

* "Seed-mediated" encompasses both flower originating seed and root derived tubers.

Fig. 70 Components of proposed Gene Flow Index (GFI) describing the propensity for successful pollen and/or seed-mediated gene flow through four possible strands: strand CPW for crop pollen-to-wild gene flow, strand CPC for crop pollen-to-crop, strand CSV for crop seed-to-volunteer and strand CSF for crop seed-to-feral, Table 3 from (Flannery et al., 2005)

Details and examples of the use of the 4 original Strands are given in (Flannery et al., 2005),

In this study, we extend the above described 4 strands again with vegetative propagation potential (tillering now additionally included in the evaluation lines for the seed and tuber mediated strands of CSV and CSF), in order to adapt the system to the reproduction biology characteristics of poplars and Miscanthus and other crops like Sorghum.

Two new strands are also added:
CGC: Consequences of Gene Flow and
CMG: Mitigation Gene Flow crop to crop, crop to wild relative and crop to feral

In summary, the following assessment of potential gene flow has been made on the system described above, extended with two new strands:

Populus, Poplar:

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) southern Europe yes, northern Europe no**	**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**
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)**	**2**
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, very low**
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) depends on flowering control**	**1-(2)-((3))**
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)**	**2**
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) depends on flowering control**	**0/1**

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	**Propensity for successful seed- and tiller mediated gene flow from commercial crop to feral**	
CSF1	**Does the crop produce seed during its cultivation? (0/1) depends on flowering control**	**0/1**
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 (limited)**
CMG2	**Mitigation with molecular safety measures** **apomixis AP, cytoplasmatic sterility CS, tandem construc TA, gene switching GS** **AP, TA, GS, (0/1) depends on flowering control**	**AP, CS, TA, GS, 1**

The consequences for the cultivation of transgenic poplars: The Dutch-Swiss-Irish scheme directs clearly to mitigation measures to be taken *before* large scale field releases and commercialization is envisaged. We know enough about biosafety of transgenic poplars, but we need to foster mitigation strategies.

Salix, Willow

1. Taxonomy

1.1. General remarks

As an introduction, first a warning word from one of the most knowledgable plant taxonomists of Europe: Why is the taxonomy of Salix so notoriously difficult ?, (Rechinger, 1992) asks and considers Salix as the ‘crux et scandalum botanicorum’: He names several reasons:

All willows are dioecious, developing male and female flowers in different individuals.
Flowers in many species are precocious, the flowers appearing before the leaves.
There is a tendency for the current year's terminal and past year's lateral branches to differ in indumentum, leaf shape and leaf sequence.
In some species, e.g. the Capreae group in addition to S. nigricans and S. hastata, parallel variation in leaf form is frequent and has misled some authors erroneously to assume hybridisation.
Hybridisation has been suspected from very early times (Smith 1805) and has been proven experimentally by Wichura (1865), Nils Heribert-Nilsson (1918), etc. Periods of over- and under-estimation of hybridisation have alternated in the past.
Fertility of Salix hybrids in many cases is not at all or only slightly reduced.
The lack of a fertility barrier between many species makes discrimination between certain species difficult.
In hybridisation one has to distinguish between primary hybrids of scattered occurrence and widespread hybrid swarms occurring where the areas of the parents overlap. In such cases it is essential to begin with the study of variation of the parental species from areas far from their overlapping areas.

1.2. Family Salicaceae

Salicaceae is a family of dioecious woody trees and shrubs with a distribution primarily in the northern hemisphere. The family comprises c. 350 species of willows and poplars, which are classically divided into two genera, *Salix* and *Populus. Salicaceae* is the only family in the order *Salicales,* which belongs to the subclass *Dillenidae* under *Magnoliophyta. Idesia polycarpa* in *Flacourtiaceae* (order *Violales)* is closely connected to *Salicaceae.* This idea was initially rejected, but has later gained ground (Cronquist, 1988; Meeuse, 1975; Miller, 1975). The coupling is supported by that fact the salicin is produced both in *Salicaceae* and *Idesia* along with some other *Flacourtiaceae,* but not by any other plants (Cronquist, 1988). Other characters shared by *Idesia polycarpa* and

Salicaceae is the presence of salicoid teeth in the leaves (Hickey & Wolfe, 1975) and several similarities in their wood anatomy (Miller, 1975).

Classic taxonomy suffers also from language barriers: the excellent taxonomic monograph of (Skvortsov, 1968), written in Russian, was largely ignored for this reason, but (Rechinger, 1992) conveyed a table which can still serve as a basis for the future molecular work: Figure 1, the synopsis taken out of (Skvortsov, 1968), the Caucasian species excluded by (Rechinger, 1992). Unfortunately, the paper of (Skvortsov, 1968) was at the time of the publication of the first edition of the Flora Europaea, where K.H. Rechinger was the contributor for Salix, not yet published, Rechinger hopes that the second edition would correct this – he already made specific suggestions.

Fig. 71 (Below) List of the European species of Salix according to the system of (Skvortsov, 1968), taken from (Rechinger, 1992)

Table 1. List of European species of *Salix* according to the system of Skvortsov (1968), Caucasian species excluded

SUBGEN. SALIX

Syn. sect. Amygdalinae Koch (1837)
S. triandra L.

Sect. Pentandrae (Borrer) C. K. Schn.
S. pentandra L.

Sect. Salix
S. alba L.
[*S.* × *rubens* Schrank]
S. fragilis L.
S. neotricha Görz

Sect. Subalbae Koidz.
(*S. babylonica* L.)

SUBGEN. CHAMAETIA (Dum.) Nasarov

Sect. Chamaetia Dum.
S. reticulata L.

Sect. Retusae A. Kerner
S. herbacea L.
S. polaris Wahlenbg.

Sect. Glaucae Pax
S. glauca L.
S. pyrenaica Gouan
S. arctica Pall.
S. retusa L.
S serpyllifolia Scop.
[*S. kitaibeliana* Willd.]

Sect. Myrtilloides L.
S. myrtilloides L.

Sect. Myrtosalix A. Kerner
S. breviserrata Flod.
S. alpina Scop.
S. myrsinites L.

SUBGEN. VETRIX Dum.

Sect. Hastatae A. Kerner
S. hastata L.
[*S. hastatella* Rech.f.]

Sect. Glabrella A. Skv.
S. crataegifolia Bert.
S. glabra Scop.

Sect. Nigricantes A. Kerner
S. myrsinifolia Salisb. (Syn. *S. nigricans* Smith) with ssp. *borealis* (Flod.) A. Skv.
S. apennina A. Skv.
S. mielichhoferi Sauter

Sect. Vetrix Dum.
S. silesiaca Willd.
S. appendiculata Vill. (Syn. *S. grandifolia* Seringe)
S. pedicellata Desf.
S. caprea L.
S. aegyptiaca L.
S. cinerea L.
S. atrocinerea Brot. (Syn. *S. cinerea* L. ssp. *oleifolia* Macreight)
S. aurita L.
S. salviifolia Brot.
S. tarraconnsis Pau. ex Fontquer
S. starkeana Willd. (Syn. *S. depressa* auctt.)

Sect. Arbuscella Seringe ex Duby
S. phylicifolia L. (Syn. *S. hegetschweileri* Heer p.p. *S. hibernica* Rech.f.) with ssp. *rhaetica* (A. Kern. ex Anders.) A. Skv.
S. basaltica Coste
[*S. cantabrica* Rech.f.]
S. arbuscula L.
S. foetida Schleich.
S. waldsteiniana Willd.

Sect. Vimen Dum.
S. viminalis L.
S. dasyclados Wimm. (*sensu* 1867 *nec prior*.)

Sect. Canae A. Kerner
S. elaeagnos Scop. (Syn. *S. incana* Schrank) with ssp. *angustifolia* (Cariot) Rech.f.

Sect. Villosae Rouy
S. lapponum L. (incl. *S. marrubiifolia* Tausch)
S. helvetica Vill.

Sect. Lanatae Koehne p.p.
S. lanata L.

Sect. Daphnella Seringe ex Duby
S. daphnoides Vill.
S. acutifolia Willd.

Sect. Incubaceae A. Kerner
S. repens L. s.l. (incl. ssp. *argentea* (Smith), *repens* L., *galeifolia* Neumann ex Rech.f.)
S. rosmarinifolia L. (incl. ssp. *angustifolia* (Wulfen) Neumann ex Rech.f.)

Sect. Helix Dum.
S. caesia L.
S. purpurea L.
S. amplexicaulis Bory & Chaubard

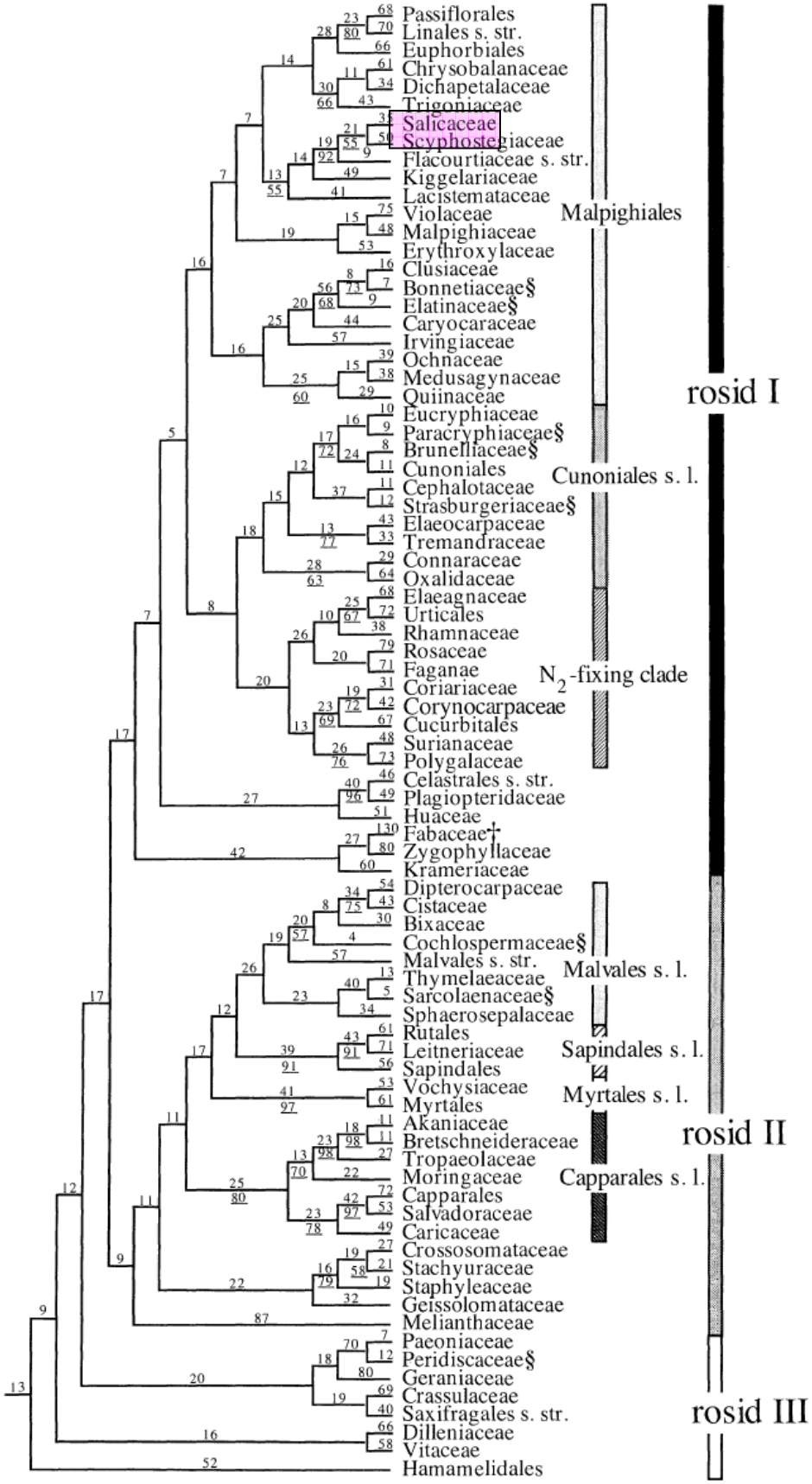
Passiflorales
Linales s. str.
Euphorbiales
Chrysobalanaceae
Dichapetalaceae
Trigoniaceae
Salicaceae
Scyphostegiaceae
Flacourtiaceae s. str.
Kiggelariaceae
Lacistemataceae
Violaceae
Malpighiaceae
Erythroxylaceae
Malpighiales
Clusiaceae
Bonnetiaceae§
Elatinaceae§
Caryocaraceae
Irvingiaceae
Ochnaceae
Medusagynaceae
Quiinaceae
Eucryphiaceae
Paracryphiaceae§
Brunelliaceae§
Cunoniales
Cephalotaceae
Strasburgeriaceae§
Elaeocarpaceae
Tremandraceae
Cunoniales s. l.
Connaraceae
Oxalidaceae
Elaeagnaceae
Urticales
Rhamnaceae
Rosaceae
Faganae
Coriariaceae
Corynocarpaceae
Cucurbitales
Surianaceae
Polygalaceae
N2-fixing clade
Celastrales s. str.
Plagiopteridaceae
Huaceae
Fabaceae†
Zygophyllaceae
Krameriaceae
Dipterocarpaceae
Cistaceae
Bixaceae
Cochlospermaceae§
Malvales s. str.
Malvales s. l.
Thymelaeaceae
Sarcolaenaceae§
Sphaerosepalaceae
Rutales
Leitneriaceae
Sapindales
Sapindales s. l.
Vochysiaceae
Myrtales
Myrtales s. l.
Akaniaceae
Bretschneideraceae
Tropaeolaceae
Moringaceae
Capparales
Salvadoraceae
Caricaceae
Capparales s. l.
Crossosomataceae
Stachyuraceae
Staphyleaceae
Geissolomataceae
Melianthaceae
Paeoniaceae
Peridiscaceae§
Geraniaceae
Crassulaceae
Saxifragales s. str.
Dilleniaceae
Vitaceae
Hamamelidales
rosid I
rosid II
rosid III

Fig. 72 The single most-parsimonious combined tree found with successive weighting. The tree has 10.271 steps (Fitch length: i.e. equal weights) with CI = 0.16 and RI = 0.38. Numbers avobe the branches are the numbers of estimated changes (ACCTRAN optimization). Underlined numbers below branches are bootstrap values; branches without an underlined number had bootstrap percentages of less than 50%. – A (left). First-branching portion of the tree, arranged with Ceratophyllaceae as the outgroup. Magnoliids form a grade composed of two major subclades (magnolid I and II) with the former sister of the eudicods. Within eudicots, ranunculids and hamamelids form a grade. The caryophyllids are sister to the asterids/rosids (for rosids, see Fig 4B). - B (right). Rosid clade. Note that the glucosinolate and nitrogen-fixing families form clades. Taxa for *rbc*L sequences were unabailable. + Nitrogen-fixing familiy outside the main nitrogen-fixing clade Fabaceae). From (Nandi et al., 1998) p. 153

Within the moderately supported Malpighiales clade (76%). Salicaceae, Scyphostegiaceae, arid Flacourtiaceae s. str. are also strongly supported as a clade (100%; with increased sampling the first two families are embedded within the last; Chase et al.. 1996). Chrysobalanaceae/Dichapetalaceae/Trigoriiacrae have moderate bootstrap support (85%), and Ochnaceae/Quiinaceae/Medusagynaceae have weak support (68%).

Comments to the figure below (Cervera et al., 2005)
158 accsessions from 25 species have been used to construct the dendrogram. A dendrogram as well as a single most parsimonious tree, ordered the Populus sections from the oldest Leuce to the latest Aigeiros, a pattern consistent with their known evolutionary relationships. A close relationship between Populus deltoides of the Aigeiros section and species of the Tacamahaca section was observed and, with the exception of Populus wilsonii, between the species of the Leucoides, Tacamahaca, and Aigeiros sections. Populus nigra was clearly separated from its consectional P. deltoides, and should be classified separately from P. deltoides. The AFLP profiles pointed out to the lack of divergence between some species and revealed that some accessions corresponded with interspecific hybrids. This molecular study provides useful information about genetic relationships among several Populus species and, together with morphological descriptions and crossability, it may help review and update systematic classification within the Populus genus.

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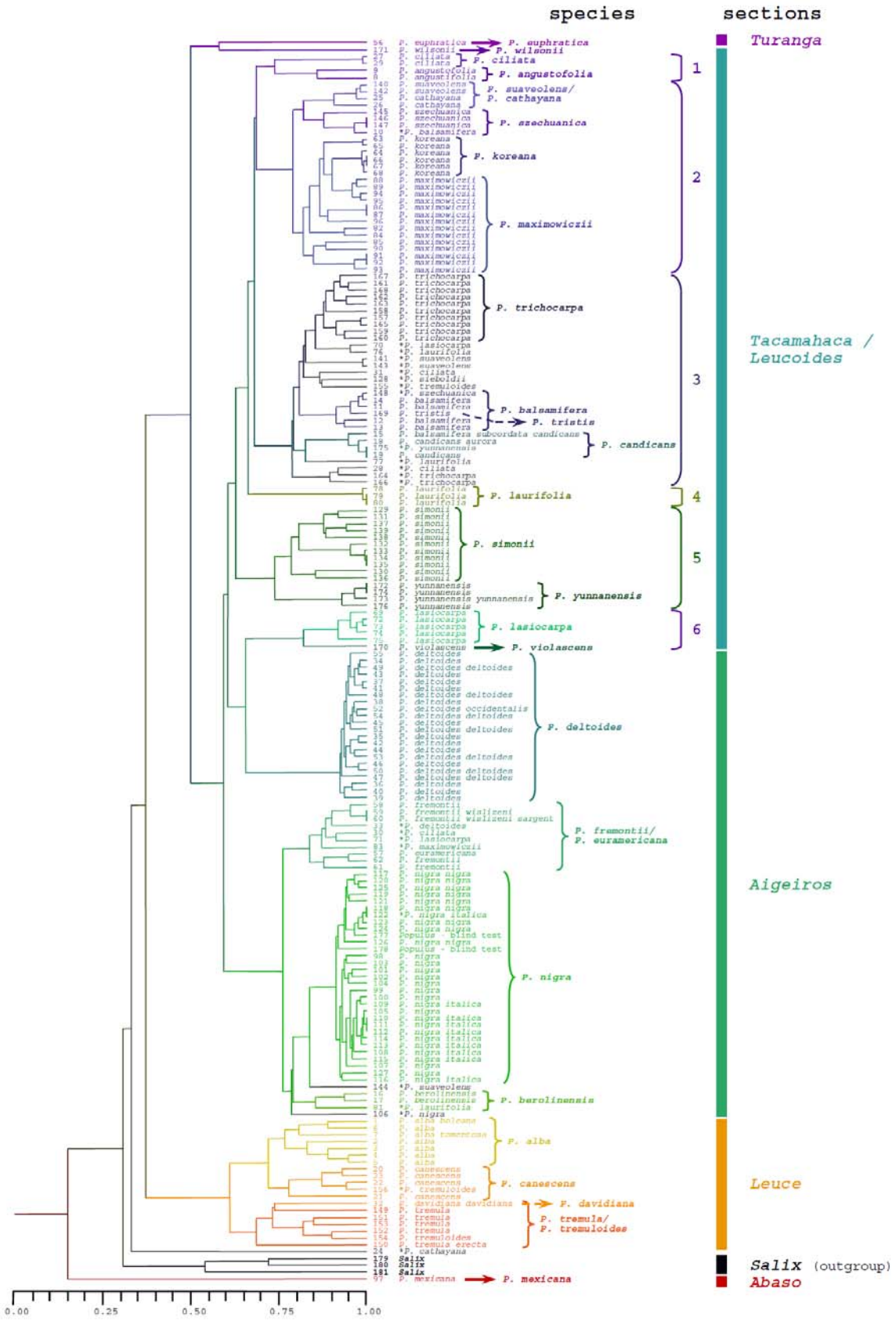

species
sections
P. euphratica
P. wilsonii
P. ciliata
P. angustifolia
P. suaveolens
P. cathayana
P. szechuanica
*P. balsamifera
P. koreana
P. maximowiczii
P. trichocarpa
*P. lasiocarpa
*P. laurifolia
*P. suaveolens
*P. ciliata
*P. sieboldii
*P. tremuloides
*P. szechuanica
P. balsamifera
P. tristis
P. balsamifera subcordata candicans
P. candicans aurora
*P. yunnanensis
P. candicans
*P. laurifolia
*P. ciliata
*P. trichocarpa
*P. trichocarpa
P. laurifolia
P. laurifolia
P. laurifolia
P. simonii
P. yunnanensis
P. yunnanensis yunnanensis
P. lasiocarpa
P. violascens
P. deltoides
P. deltoides deltoides
P. deltoides occidentalis
P. deltoides deltoidos
P. fremontii
P. fremontii wislizeni
P. fremontii wislizeni sargent
*P. deltoides
*P. ciliata
*P. lasiocarpa
*P. maximowiczii
P. euramericana
P. fremontii
P. nigra nigra
P. nigra italica
P. nigra nigra
Populus - blind test
P. nigra nigra
Populus - blind test
P. nigra
P. nigra italica
*P. suaveolens
P. berolinensis
P. berolinensis
*P. laurifolia
*P. nigra
P. alba boleana
P. alba tomentosa
P. alba
P. alba
P. alba
P. canescens
P. canescens
P. canescens
*P. tremuloides
P. canescens
P. davidiana davidiana
P. tremula
P. tremula
P. tremula
P. tremuloides
P. tremula erecta
*P. cathayana
Salix
Salix
Salix
P. mexicana
P. euphratica
P. wilsonii
P. ciliata
P. angustifolia
P. suaveolens/
P. cathayana
P. szechuanica
P. koreana
P. maximowiczii
P. trichocarpa
P. balsamifera
P. tristis
P. candicans
P. laurifolia
P. simonii
P. yunnanensis
P. lasiocarpa
P. violascens
P. deltoides
P. fremontii/
P. euramericana
P. nigra
P. berolinensis
P. alba
P. canescens
P. davidiana
P. tremula/
P. tremuloides
P. mexicana
1
2
3
4
5
6
Turanga
Tacamahaca /
Leucoides
Aigeiros
Leuce
Salix (outgroup)
Abaso
0.00
0.25
0.50
0.75
1.00

Fig. 73 Dendrogram of Populus and Salix accessions, constructed from AFLP fragment similarities (Dice coefficient), with the UPGMA clustering method, and based on AFLP markers resolved by five primer combinations (EcoRI+ATA/MseI+ACAA, EcoRI+ ATA/MseI+ACAC, EcoRI+ATA/MseI+ACAG and EcoRI+ ATA/MseI+ACAT, EcoRI+AAA/MseI+ACAT). Accessions marked with an asterisk are potentially mislabeled species or hybrids (see text and Table 2). Species are marked by brackets and arrows, whereas lines group sections. Fig. 1 from (Cervera et al., 2005)

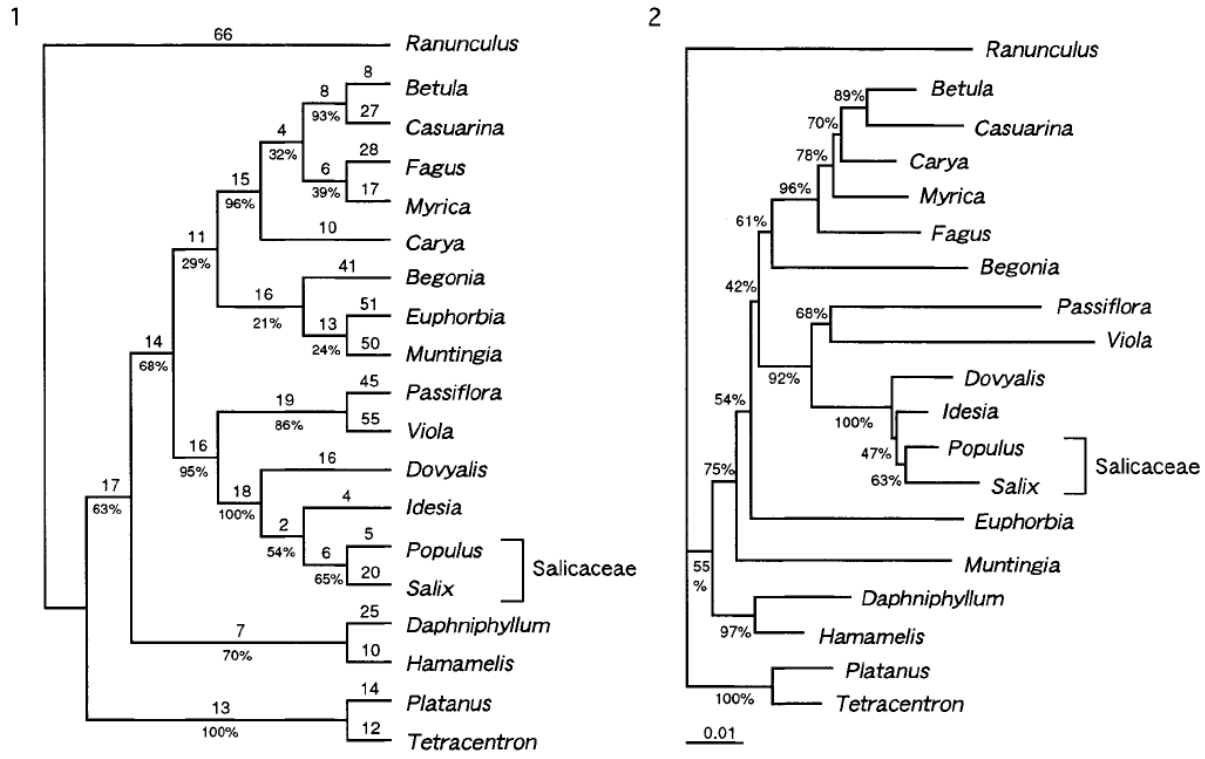


Fig. 74 Relationships of Salicaceae and putative related families based on *rbcL* data. 1. The single minimum length Fitch parsimony tree. Branch length (number of nucleotide substitutions) is indicated above the branches and bootstrap values (100 replicates) are indicated below them. 2. The tree obtained from neighbor-joining method. Numbers near branches are bootstrap values (100 replicates). Scale of branches indicates numbers of nucleotide substitutions per sites calculated by Kimura's two-parameter method (Kimura, 1980). From (Azuma et al., 2000)

1.2. The genus Salix

Salix L. is by far the largest of the 2–4 genera of the family Salicaceae. Systematic treatments of *Salix* have varied extensively (Table 1). Salicaceae was divided into *Salix* and *Populus* when it was originally described by (Linnaeus, 1753). Systematic treatments within *Salix* have also disagreed with one another. (Kimura, 1928) divided *Salix* into two subgenera, and (Skvortsov, 1968) into three. Later, (Kimura, 1988) returned *Chosenia* and *Toisusu* as *Salix* subg. *Pleuradenia* Kimura.

Such taxonomic discordance in systems of *Salix* at the generic and subgeneric level are caused by scarceness of informative morphological characters that can be used for

systematic studies. Although many important characters for systematics of angiosperms are chiefly obtained from flowers, such floral characters are limited in Salicaceae because of their extremely reduced flowers. It is necessary, therefore, to seek other sources of information for reexamining the classification of *Salix.*

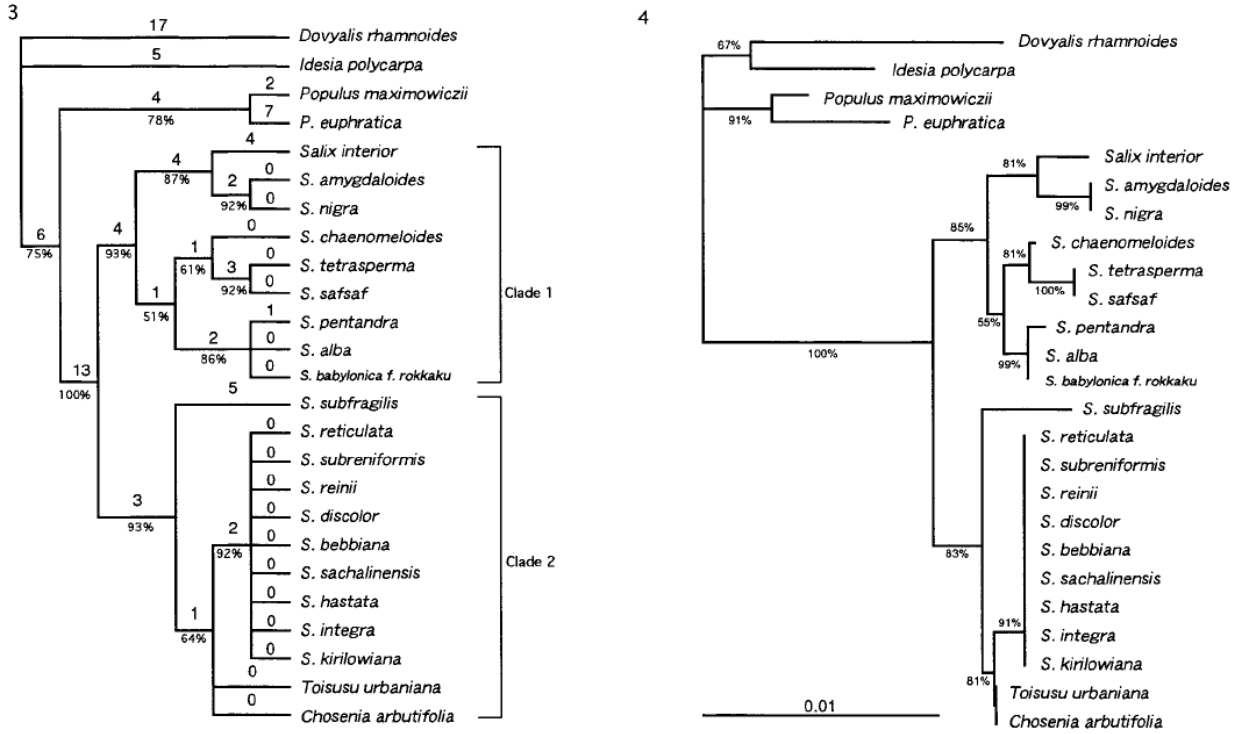


Fig. 75 Relationships of Salicaceae based on *rbcL* data. 3. The single minimum length Fitch parsimony tree. Branch length (number of nucleotide substitutions) is indicated above the branches, and bootstrap values (100 replicates) are indicated below them. 4. The tree obtained from neighbor-joining analysis. Numbers near branches are bootstrap values (100 replicates). Scale of branches indicates numbers of nucleotide substitutions per sites calculated by Kimura's two-parameter method (Kimura, 1980), from (Azuma et al., 2000).

(Azuma et al., 2000) demonstrates with the below plate how controversial on the species and subgenus level Salix species systematics was handled:

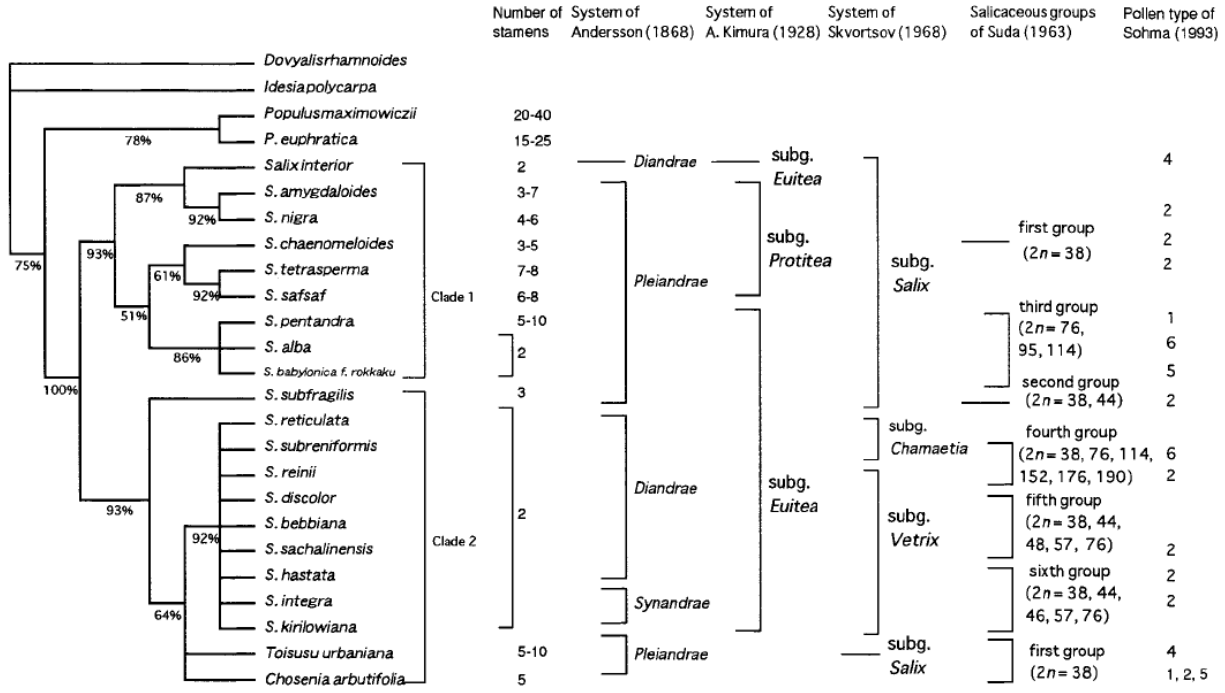


Fig. 76 Relationships of grouping in previous studies and the single minimum length Fitch parsimony tree obtained for taxa of Salicaceae based on *rbcL* sequences. Bootstrap values are indicated below branches. From (Azuma et al., 2000)

This study revealed that there are two clades in *Salix.* The authors compared these two clades with taxonomic systems proposed so far. Andersson (1868) divided *Salix* into three groups mainly based on stamen characters: *Pleiandrae* Anderss, which has more than two stamens (but *S. alba* and *S. babylonica* included in this group have two stamens), *Diandrae* Anderss, which has two separate stamens, and *Synandrae* Anderss, which has two connate stamens. Our molecular tree suggested that Pleiandrae were paraphyletic and *Diandrae* were polyphyletic and that *Synandrae* formed a monophyletic group with most of *Diandrae* except for S. interior (Fig. 6).
With regard to stamens, Skvortsov (1968) considered that the reduction of stamen number from more than two stamens to two might occur in three lineages, yielding the following three groups: (1) *Salix* sect. *Longifoliae*, (2) *Salix* sect. *Salix* and sect. *Subalbae* Koidz., and (3) *Salix* subg. *Chamaetia* and subg. *Vetrix.* In our molecular data (Fig. 6), subg. *Chamaetia* and subg. *Vetrix* formed a monophyletic group, while *S. babylonica* f. *rokkaku* of sect. *Subalbae* and *S. alba* of sect. *Salix* appeared in another clade, though they formed a trichotomy with *S. pentandra*, which has more than two stamens. These two groups and *S. interior* of sect. *Longifoliae*, the third twostamen group, appeared independently. Therefore, our molecular data support Skvortsov's viewpoint concerning the evolution of stamens.

No morphological, anatomical, palynological, or cytological characters that support the two phylogenetic groups of *Salix* obtained in the results of (Azuma et al., 2000) were found in any previous studies. Therefore, one should examine *Salix* for additional characters suggesting phylogenetic relationships. (Azuma et al., 2000) demonstrated using cpDNA that there are two clades in *Salix.* However, extensive hybridization is known and cytoplasmic capture has been discovered in the Salicaceae (Brunsfeld et al., 1992). Thus, it is probably difficult to discuss the exact history of evolution by using cpDNA alone. Therefore, one should also examine nuclear markers like nrDNA to reveal the history of evolution in the Salicaceae.

(Azuma et al., 2000) achieved extensive phylogenetic knowledge of the Salicaceae using the *rbcL* gene. These results alone do not reveal the evolution of the Salicaceae, and it is necessary to examine other molecular sources to confirm the results.

A really instructive diagram with a comparison of morphological an genetic characters is given by (Hardig et al., 2000), it clearly demonstrates the improved accuracy of numerical Taxonomy with the help of genetic analysis.

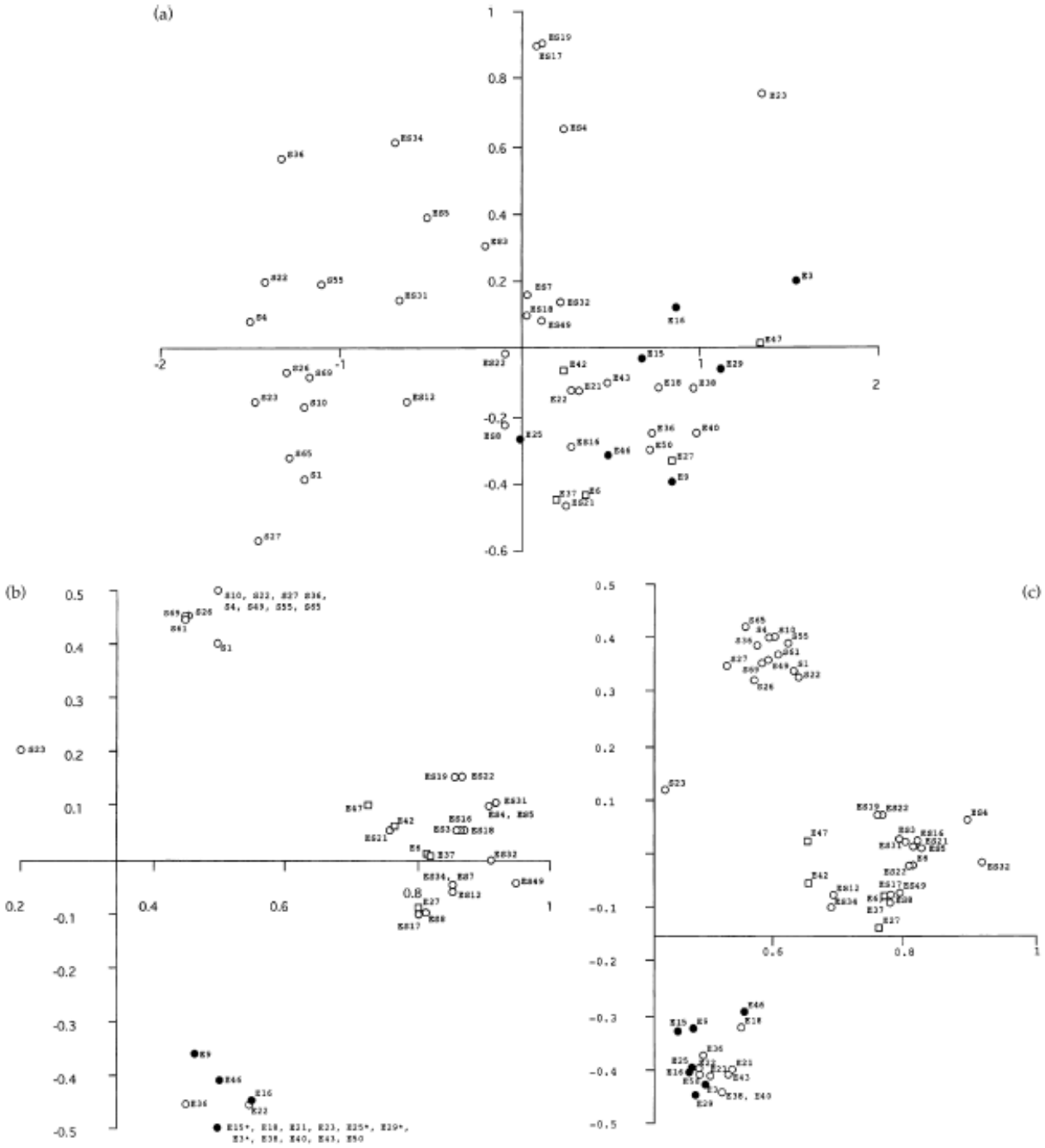


Fig. 1 Plot of *Salix* specimens by first- and second-factor scores derived from PCA of: (a) morphology characters; (b) original 20 RAPD markers; (c) 43 RAPD markers. Filled circles represent the e-type cpDNA; hybrids misidentified in the field as *S. eriocephala* are indicated by squares (see text).

Fig. 77 Plot of *Salix* specimens by first- and second-factor scores derived from PCA of: (a) morphology characters; (b) original 20 RAPD markers; (c) 43 RAPD markers. Filled circles represent the e-type cpDNA; hybrids misidentified in the field as *S. eriocephala* are indicated by squares (see text).

Many of the following papers on genetics of poplar can also be of relevance for the genus Salix: (Bekkaoui et al., 2003; Brunner et al., 2004; Brunner & Nilsson, 2004; Busov et al., 2003; Cervera et al., 2005; Choi et al., 2006; Christopher et al., 2004; Cole, 2005; Cseke et al., 2005; Djerbi et al., 2005; Johansson et al., 2002; Lescot et al., 2004; Leseberg et al., 2006; Martin & Kohler, 2004; Martin et al., 2004; Moreau et al., 2005; Nicole et al., 2006; Nishiguchi et al., 2002; Park et al., 2004; Ralph et al., 2006; Robinson et al., 2005; Sampedro et al., 2006; Sjodin et al., 2006; Smith & Campbell, 2004; Sterck et al., 2005; Sterky et al., 2004; Stokstad, 2006; Strauss & Martin, 2004; Street et al., 2006; Teeri & Brumer, 2003; Tsai & Hubscher, 2004; Tschaplinski et al., 2006; Tuskan et al., 2006; Tuskan et al., 2004; Yin et al., 2004; Yin et al., 2002; Zalesny & Wiese, 2006; Zhang et al., 2004; Zhang et al., 2005; Zhao et al., 2005a) and many others poplar and willow genomics has made enormous progress from 2002 onwards: Trees present a life form of paramount importance for terrestrial ecosystems and human societies because of their ecological structure and physiological function and provision of energy and industrial materials. The genus populus is the internationally accepted model for molecular tree biology. It should also serve for prospecting molecular work in the genus Salix.

Although efforts to identify *Populus* as a model tree began long before sequencing a tree genome was a possibility, the choice of poplar was ideal in that the genome size is small, 550 Mbp. This is similar in size to the rice genome, only 4 times larger than the genome of Arabidopsis, yet 40 to 50 times smaller than the genome of pine, (Wullschleger et al., 2002).

Another effort to come to terms with genes and phylogeny concentrating on the comparison with two well known genomes Oryza and Arabidopsis has been recently summarized in (Tuskan et al., 2006) with the following figures:

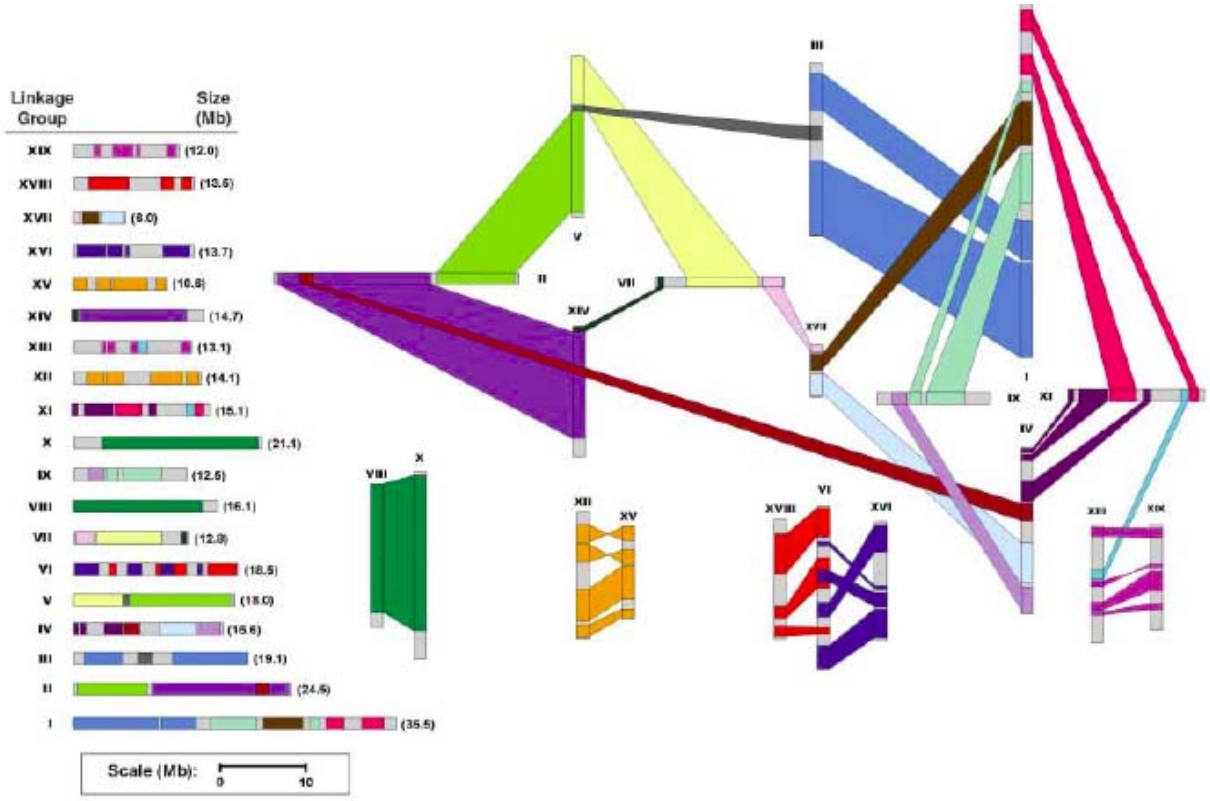


Fig. 78 Chromosome-level reorganization of the most recent genome-wide duplication event in Populus. Common colors refer to homologous genome blocks, presumed to have arisen from the salicoid-specific genome duplication 65 Ma, shared by two chromosomes. Chromosomes are indicated by their linkage group number (I to XIX). The diagram to the left uses the same color coding and further illustrates the chimeric nature of most linkage groups, from (Tuskan et al., 2006)

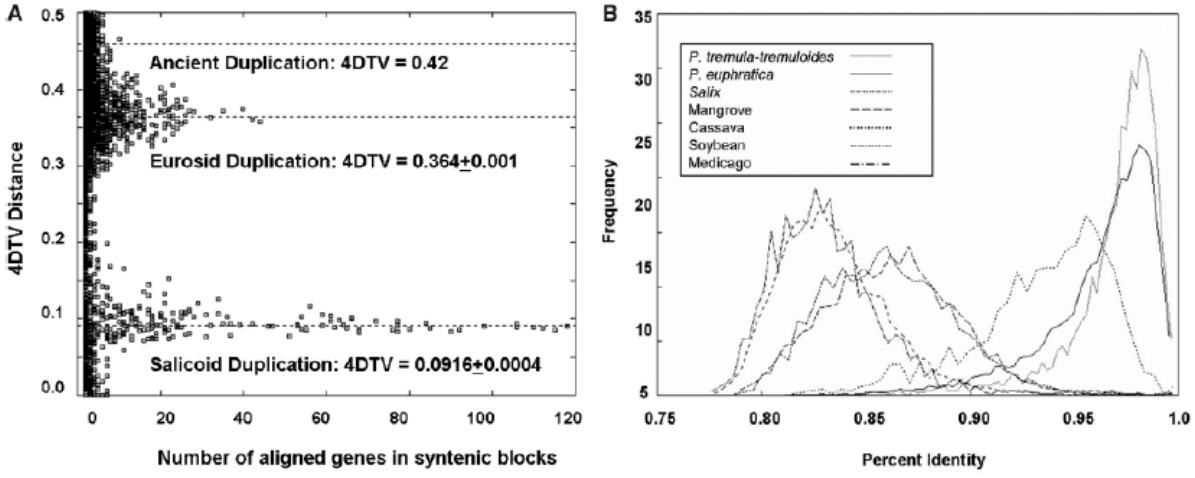


Fig. 79 (A) The 4DTV metrics for paralogous gene pairs in Populus-Populus and Populus-Arabidopsis. Three separate genome-wide duplications events are detectable, with the most recent event contained within the Salicaceae and the middle event apparently shared among the Eurosids. (B) Percent identity distributions for mutual best EST hit to Populus trichocarpa CDS, from (Tuskan et al., 2006).

As a whole, the systematics of Populus and Salix will undergo still more revisions, here the ‘state of error’ given by the OECD document (OECD, 2001b), based on (Eckenwalder, 1996)

A visualized result of the new relationships in Populus and Salix is again given by (Cervera et al., 2005).

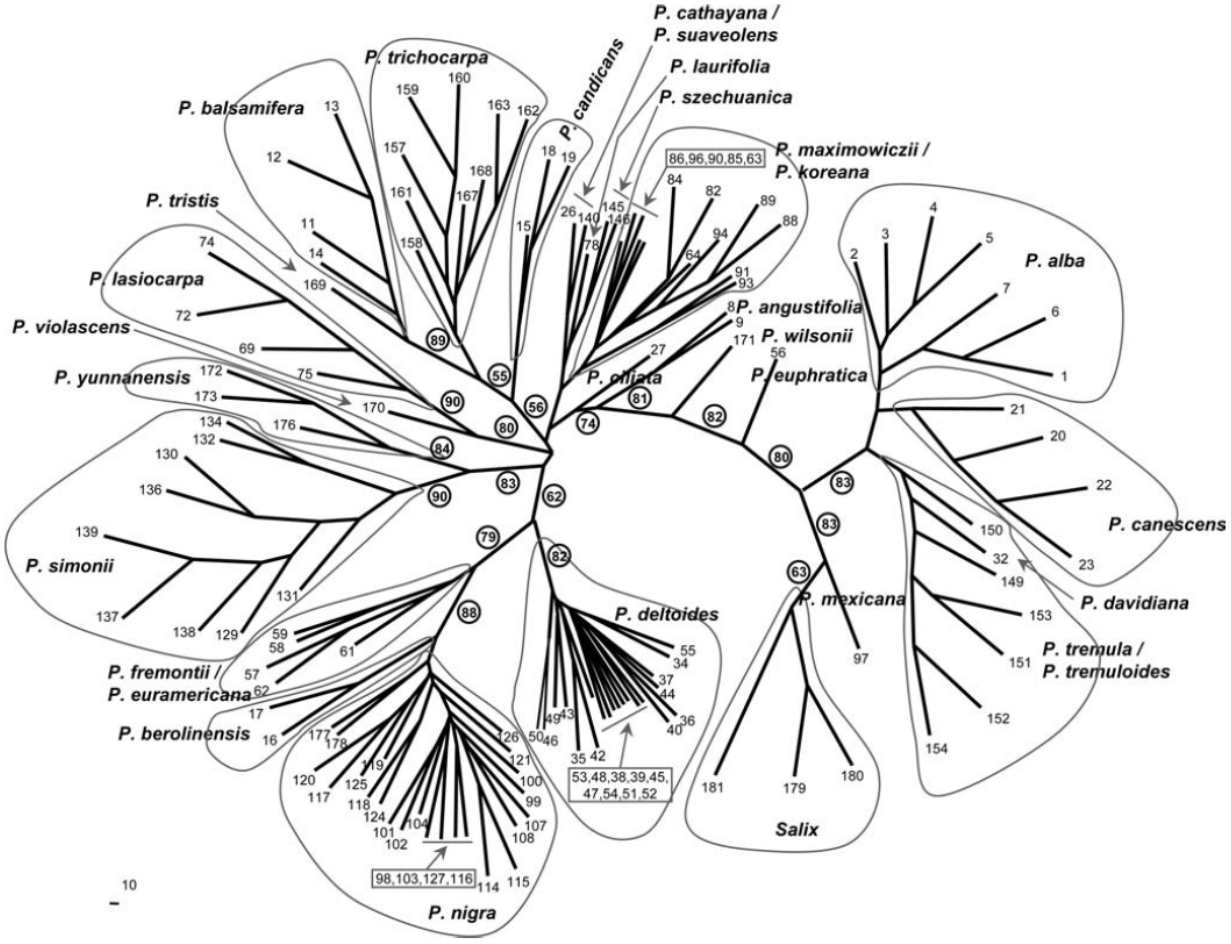


Fig. 80 The single most parsimonious bifurcating unrooted tree, based on the Wagner method, representing the phylogeny of Populus, including a branch for Salix (179-181). Plain and circled numbers correspond to accession codes (Table 2) and bootstrap values (only those above 50% are shown for main branches, grouping several species), respectively. Fig 2 from (Cervera et al., 2005).

A word of caution: The correlation between morphology and genetics is often strikingly disparate, as shown by (Hardig et al., 2000)
When they plotted individuals in two dimensions, using stipule width and length values, they recovered a linear distribution of individuals, with most field-identified hybrids occupying intermediate positions (Fig. 11). However, when molecular rDNA and chemistry data are superimposed on to this pattern, incongruities between the data sets become apparent. Specimen E25 occurs in a position that is unmistakably intermediate to either parent (arrowed in Fig. 11), yet it possesses typical S. eriocephala genetics and

chemistry. Apparent intermediacy of an individual, as is evident in specimen E25, might simply be expression of a not necessarily extreme degree of native genetic diversity within S. eriocephala. In addition, hybrid intermediacy in morphological characters assumes that all characters are expressed in an additive fashion within hybrids, but as we have shown in this study (and reviewed in Rieseberg & Ellstrand 1993), this assumption is often unwarranted. *Therefore, there is no reason to take intermediacy as evidence of hybridity.*

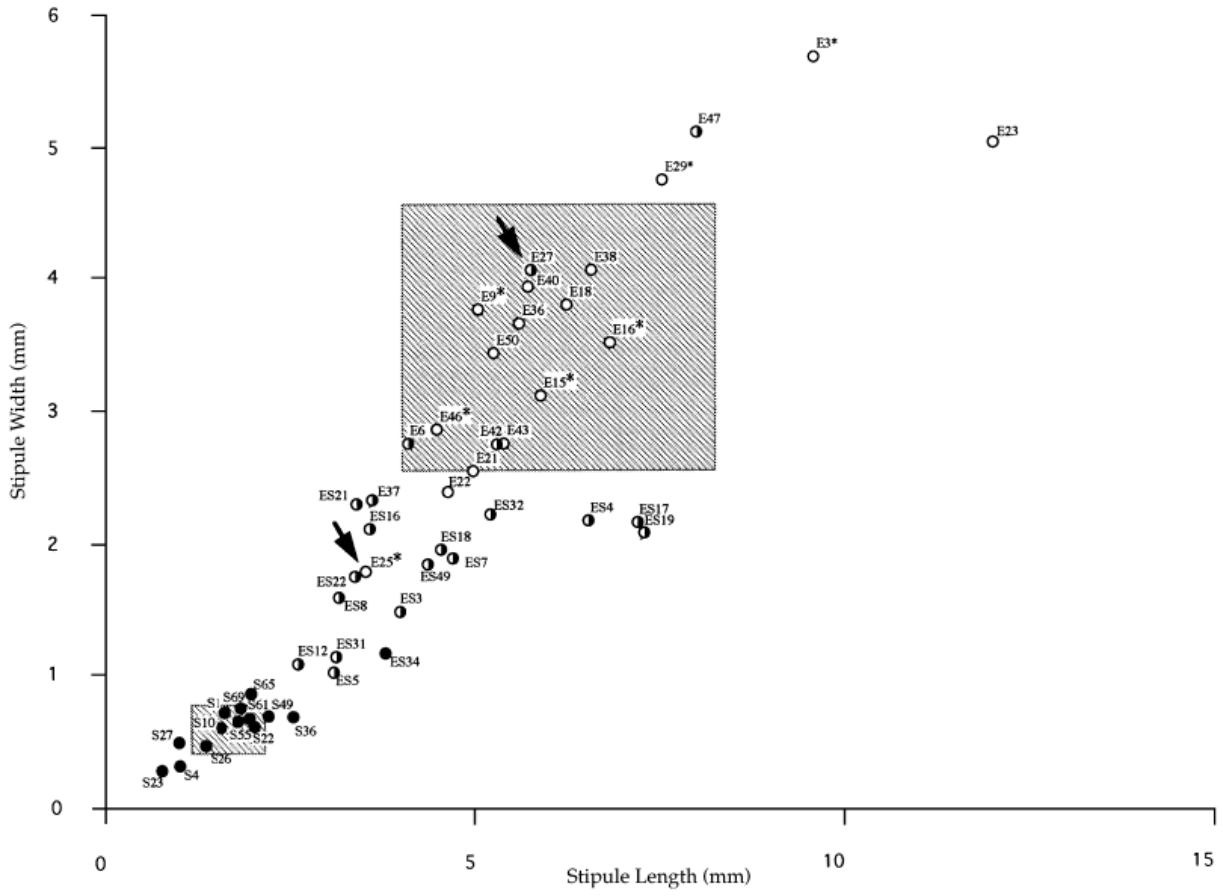


Fig. 81 Scatter diagram of specimens plotted by stipule length and stipule width. An asterisk (*) represents the e-type cpDNA. Circle shading indicates the rDNA genotype: white, –e/e; black, s/s; half-white and half-black, –e/s (see text). Hatched boxes indicate two standard deviations about the means for pure parents, determined by the second ML iteration. Arrows indicate specimens discussed in the text. From (Hardig et al., 2000)

Another rather surprising fact is that e.g. Salix alba and Salix fragilis, usually considered to hybridize freely and often, reveals with close genetic scrutiny that true hybrids in those populations are rare. (Triest et al., 2000) A thorough screening of full-sib progenies of interspecific controlled crosses was made to select homologous amplification products. The selected amplified products proved to be useful in a principal coordinate analysis for the estimation of variability of hybrid progenies. On the basis of genetic similarities and ordination analysis, a method for the identification of clones in the field was established

using presumed pure species and presumed introgressants. The chosen reference clones were checked against additional European samples of putative pure species to ensure the reliability of the method beyond a regional scale. The RAPDs suggested that both species have kept their gene pools well separated and that hybridization actually does not seem to be a dominating process. Molecular markers do not always follow the morphological traits or allozyme data.

5. Reproduction biology

Basically, all willows are dioecious, developing male and female flowers in different individuals. Flowers in many species are precocious, the flowers appearing before the leaves.
(Tuskan et al., 2006) described the genetic basis of the reproduction biology of populus as follows:
Unlike Arabidopsis, where predominantly self-fertilizing ecotypes maintain low levels of allelic polymorphism, Populus species are predominantly dioecious, which results in obligate outcrossing. This compulsory outcrossing, along with wind pollination and wind-dispersed plumose seeds, results in high levels of gene flow and high levels of heterozygosity (that is, within individual genetic polymorphisms). This is true also for Salix.

But demographic and biogeographic studies show a differenciated picture in time and space from species to species, and some variation can even be detected within a single species:

(Falinski, 1998) studied the case of Salix myrsinites, which is bisexua or monosexual according to various conditions and life cycle phase:
The aims of the study were to describe the phenomenon of androgyny (a change in the sexual behaviour of individuals) in *Salix myrsinifolia*, and to consider the implications of androgyny for the sex structure of local populations and for the sex structure of the species across a larger geographical range. Field surveys of the sexual behaviour of individuals and populations of *Salix myrsinifolia* were carried out over nine years (1989–1997) in an area of 40,000 km_2 of NE-Poland. More detailed studies were performed on populations in Polana Bial/owieska (the Bial/owiez˙a Clearing) in the Bial/owiez˙a Primeval Forest, in the Bial/ystok area, in the Biebrza Valley and in the experimental garden and laboratory of the Bial/owiez˙a Geobotanical Station. The bisexuality of *Salix myrsinifolia* is basically expressed through the development of a number of different forms of catkin that are intermediate between those that are entirely male and those that are entirely female. Flowers on bisexual catkins are fully developed, as on monosexual ones, but the male segment usually develops prior to the female one. With the exception of one form, a clear partitioning between the male and female parts of the catkin remains. This partitioning of the genders mainly takes place transversely, but in one case longitudinally. It is thus usually possible to speak of the division of a bisexual catkin into male and female sectors. In all three populations studied in detail, marked shrubs,

on which bisexuality had been noted at the beginning, retained this trait throughout the 9-year observation period. However, there were changes in both the expression of bisexuality, i.e. the frequency of bisexual and monosexual catkins, and in the frequency of different patterns of bisexual catkin. Only bisexual individuals with a clear prevalence of female features retained their character. In the two larger populations studied, many originally monosexual individuals became bisexual during the study period. In the south-western part of the range of *S. myrsinifolia* in NE-Poland all local populations were characterized by the presence of bisexual individuals and are thus polygamous. There was a close association between the presence of *S. myrsinifolia* and the degree of ruderalization of habitats. The degree of polygamy in a population was also significantly correlated with ruderalization. It is concluded that androgyny and polygamy may be favoured not only by changes in environmental conditions, but also by the particular pressures to which individuals and populations of a species may be subject at the edge of its geographical range.

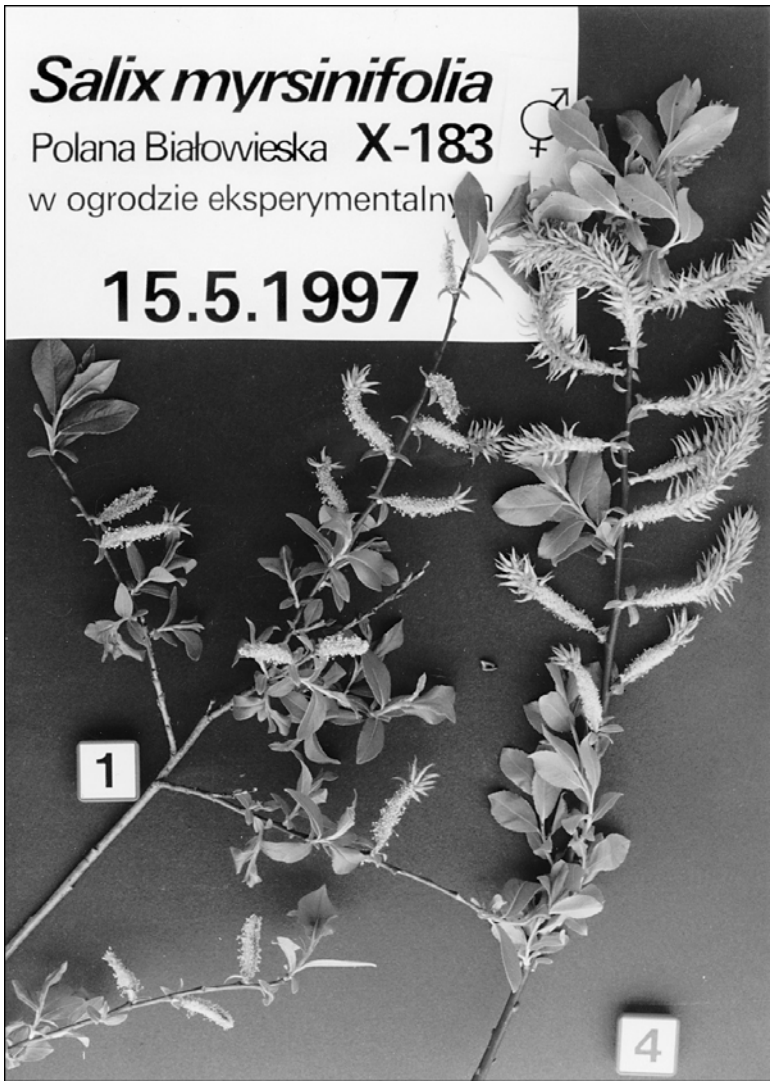
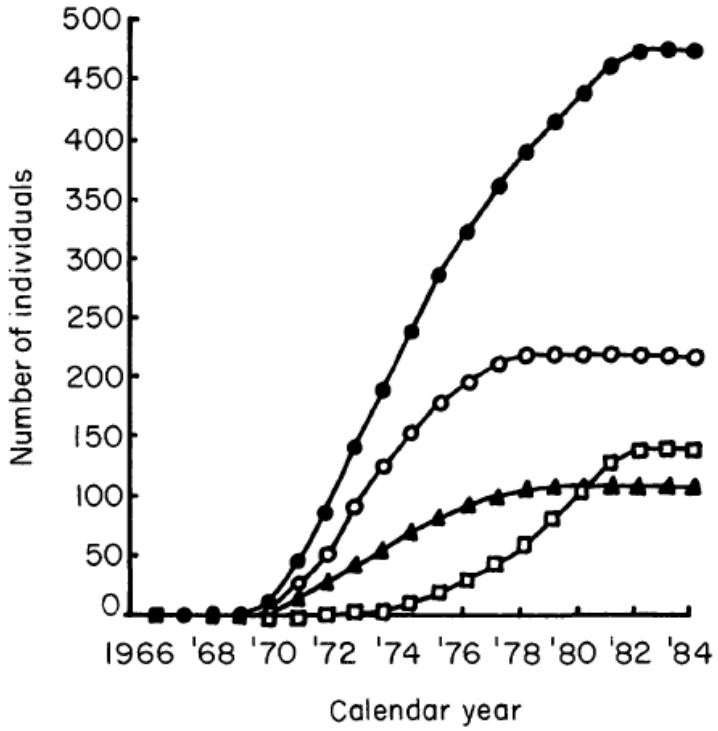


Fig. 82 Branches from two shrubs of S. myrsinifolia with bisexual catkins. The original plants grew beside a forest road in the Bialowiez˙a Forest and were observed for seven years (1989–1995). ranches were later transplanted to the experimental garden. The photograph was taken two years after transplanting (Photo J.B. Falinski). From (Falinski, 1998)

In a case study on Salix caprea by (Alliende & Harper, 1989) analyse demographic data over a test area and over a long time span, the reproduction strategy revealed to be dependent on time and space within the population. In summary, reproduction in willows is adapted to the many pioneer species, which get lots of advantage with a highly adaptable and flexible biology.

This is shown in two diagrams, based on an extensive demographic study by (Alliende & Harper, 1989), they show the full dynamics of reproduction biology, and do not need furher explanation. It is also a blatant demonstration of how difficult field recognition is in this genus, allthemore also leaf morphology changes dramatically with the age of the branches.



. The growth of the population of *Salix cinerea* at Newborough Warren, Anglesey, Wales. Females (○), males (▲), plants that did not flower during the study (□) and total plants (●).

Fig. 83 Caption see above, from (Alliende & Harper, 1989)

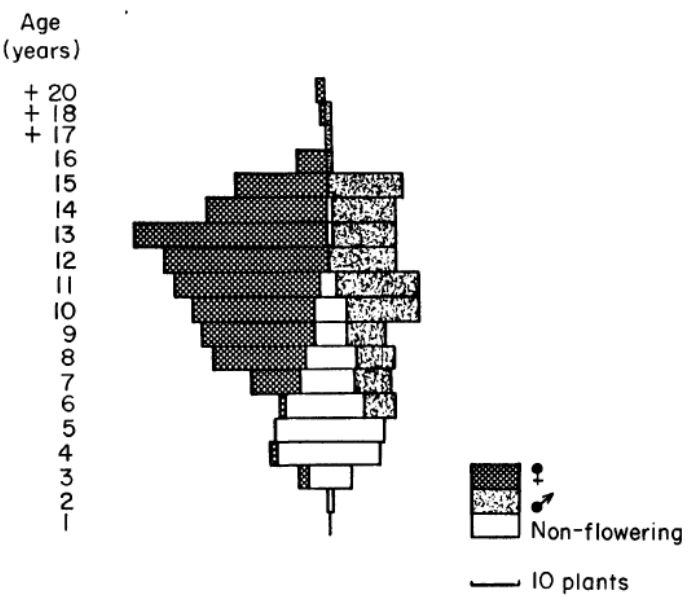


Fig. 84 Pyramid of age and sex of the population of Salix cinerea at the Newborough Warren, Anglesey, Wales in 1984. Female plants, male plants and non-flowering (juvenile) plants, from (Alliende & Harper, 1989)

Salix, just as populus, is known for its interplay between hybridization and clonal reproduction. This has been studied for example in the case of S. eriocephala, S. exigua, S. eriocephala and S. petiolaris by (Salick & Pfeffer, 1999):
Clonal reproduction may contribute to plant evolution either by affecting population biology or by allowing partially sterile individuals (e.g., hybrids) repeated opportunities to reproduce sexually. The interplay of hybridization and clonal reproduction was first proposed by (Stebbins, 1950), but previously has not been tested experimentally. Alternative models for the effects of hybridization include speciation, introgression, and swamping. *Salix* spp. were chosen to test comparatively these hypotheses because they are easily hybridized and cloned. Over 500 separate crosses and backcrosses were made and over 6000 separate plants were measured in field experiments and statistically compared for significance to both evolutionary theory and plant breeding for biomass production. The F_1 hybrids in this study always equalled, and in the case of the hybrid PR, outperformed their parents in vegetative parameters. It seems likely that even without reproducing sexually, these F_1 hybrids could exist as successful individuals (*sensu* (Stebbins, 1950). However, it also seems likely that they would sexually reproduce: three of four F_1 hybrids studied (RX, RP, and PR) equalled or surpassed their parents in sexual parameters when crossing with at least one other accession. Of the alternative models, experimental data suggest that introgression would be the most likely outcome of a hybridization event. The hybrid XR, however, was partially sterile and performed poorly when crossing with all other accessions in its group except *S. exigua* (pistillate parent). Thus, this hybrid may fit Stebbins' model of a partially sterile yet vegetatively vigorous plant that can exist as a successful individual and make some contribution to interspecific gene flow over time. This is the first experimental study to confirm the evolutionary importance of clonal reproduction coupled with hybridization.

However, distinguishing any of these evolutionary pathways would be difficult in nature using morphological techniques, as interspecific hybrids tend to resemble their pistillate parents in terms of leaf shape.

6. Risk assessment of transgenic salices

In a review on transgenic trees as tools in tree and plant physiology (Herschbach & Kopriva, 2002) the following summary scheme is helpful

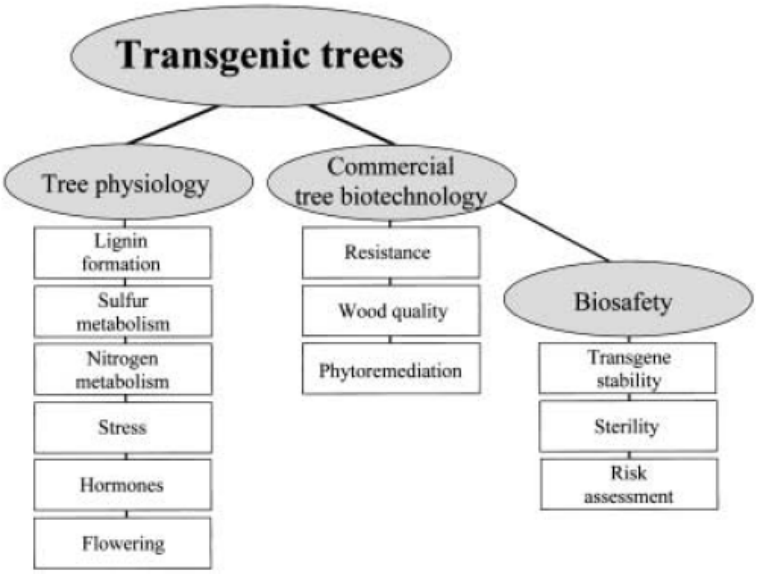


Fig. 85 Transgenic trees in tree physiology and biotechnology from (Herschbach & Kopriva, 2002)

6.3. Risk assessment: some characteristics for transgenic trees and poplar in particular

Pollen mediated gene flow is important, but vegetative propagation has to be considered as well, as already stated by the OECD consensus paper (OECD, 2001b) for Populus, which reacts in many cases like Salix:

Domestication of transgenes can provide new avenues to promote biosafety.
In short, transformation in poplar is extremely reliable and there are diverse and promising means for improving biosafety, but considerable time, institutional commitments and public–private partnerships are required to deliver them to society. There are other ways and means to mitigate gene flow, but unfortunately, the long breeding cycles make things more difficult. Promising pathways of mitigation have been proposed by (Al-Ahmad et al., 2006a; Al-Ahmad et al., 2004, 2006b; Al-Ahmad & Gressel, 2005, 2006; Gressel & Al-Ahmad, 2005): They developed tandem constructs, which would avoid or at least reduce considerably the spread of transgenes, the concept

has been proven to produce stable gene constructs and sustainable mitigation: Transgenic mitigation (TM), where a desired primary gene is tandemly coupled with mitigating genes that are positive or neutral to the crop but deleterious to hybrids and their progeny.
This was tested experimentally by the team of Gressel from Rehovot in Israel as a mechanism to mitigate transgene introgression. Dwarfism, which typically increases crop yield while decreasing the ability to compete, was used as a mitigator. A construct of a dominant ahasR (acetohydroxy acid synthase) gene conferring herbicide resistance in tandem with the semidominant mitigator dwarfing Δ gai (gibberellic acid-insensitive) gene was transformed into tobacco (Nicotiana tabacchum). The highest reproductive TM fitness relative to the wild type was 17%. The results demonstrate the suppression of crop–weed hybrids when competing with wild type weeds, or such crops as volunteer weeds, in seasons when the selector (herbicide) is not 273 used. The linked unfitness would be continuously manifested in future generations, keeping the transgene at a low frequency.

Salix, willow

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) southern Europe yes, northern Europe no	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
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)	2
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, very low
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) depends on flowering control	1-(2)-((3))
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)	2
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) depends on flowering control**	**0/1**
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	**Propensity for successful seed- and tiller mediated gene flow from commercial crop to feral**	
CSF1	**Does the crop produce seed during its cultivation? (0/1) depends on flowering control**	**0/1**
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 (limited)**
CMG2	**Mitigation with molecular safety measures** **apomixis AP, cytoplasmatic sterility CS, tandem construc TA, gene switching GS** **AP, TA, GS, (0/1) depends on flowering control**	**AP, CS, TA, GS, 1**

The consequences for the cultivation of transgenic poplars: The Dutch-Swiss-Irish scheme directs clearly to mitigation measures to be taken *before* large scale field releases and commercialization is envisaged. We know enough about biosafety of transgenic poplars, and we need urgently to expand our knowledte to the genus Salix, and then, knowing more about reproduction modes and gene flow, we need to foster mitigation strategies.

Bibliography of Salix from the Web of Science, 30. January 2007
http://www.botanischergarten.ch/EPOBIO-Salix/Bibliography-EPOBIO-Salix.pdf

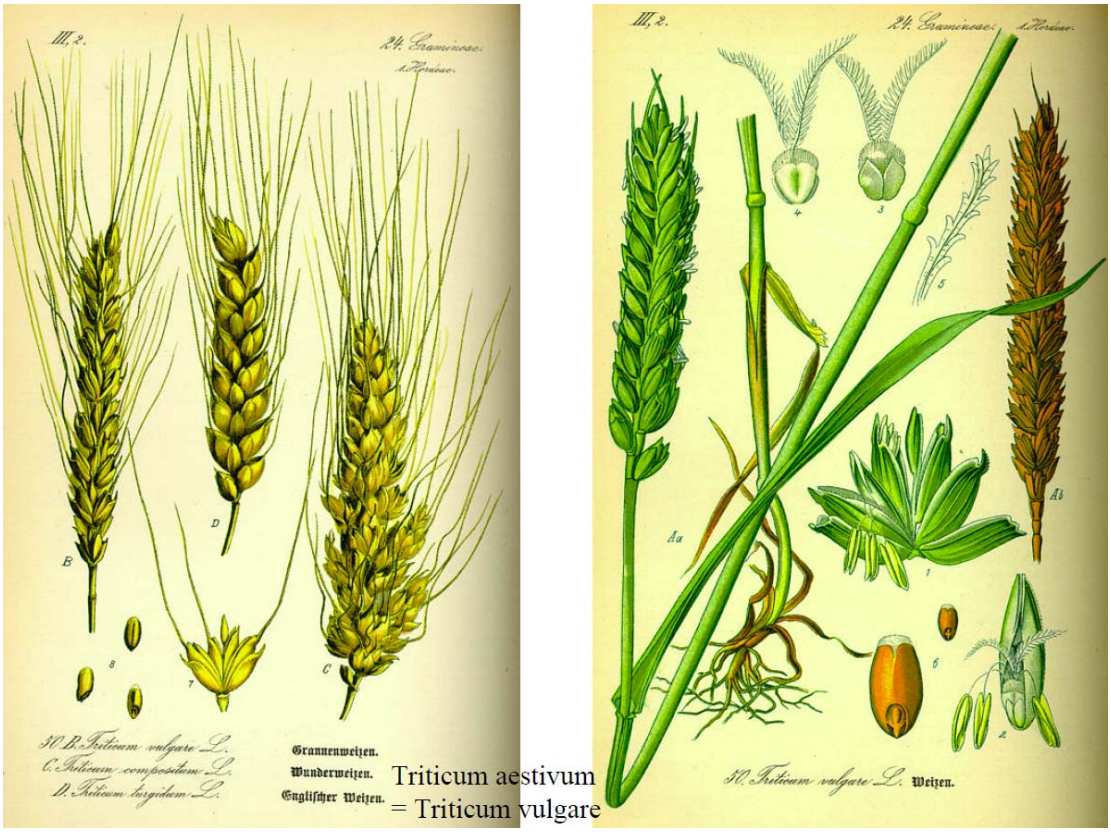
Triticum, Wheat

1. Introduction

This is an update of the most recent literature on the taxonomy of wheat, its reproduction biology and gene flow, related to biosafety of future field experiments and commercialisation of new wheat traits. In most cases, only peer reviewed literature is cited and documented, with an emphasis on publications based on experimental data.

2. Taxonomy

Triticum aestivum, bread wheat, belongs to the order *Poales* (*Glumiflorae*), family *Poaceae* (*Gramineae*), tribe *Triticeae*, genus *Triticum*. The tribe *Triticeae* consists of 18 genera which are divided into two sub-groups, the *Triticinae* and the *Hordeinae*. The major genera in the sub-group *Triticinae* are *Triticum, Aegilops, Secale, Agropyron* and *Haynaldia* (Odenbach, 1985), (Zeller & Friebe, 1991) (Körber-Grohne, 1988).
Plants of the genus *Triticum* are annuals with spring or winter forms. They show the following morphological features: short ligule and spikelets that are sometimes hairy, and a smooth, bald, usually hollow culm, 0.7-1.6 metre in height. Pithy filling is less common than a hollow culm. The ears have a brittle or tough rachis. Generally they are four-sided. The spikelets have two to five florets. Each floret can produce one grain (caryopsis), i.e. is distichous. The glumes are keeled, on the upper side for example in *T. aestivum*, with serrated lemmas, long and either bearded or unbearded. Grains are loosely enclosed (naked wheat) and easily threshed. The rachilla has thin walls and does not disarticulate on maturity. In case of *T. aestivum* ssp. *spelta* (spelt wheat) the grains are hulled by the spelta. For this reason they cannot be dropped during the process of threshing (Garcke, 1972; Geisler, 1991). *T. aestivum* is a cereal of temperate climates. The northern limit of wheat cultivation in Europe lies in southern Scotland (60° latitude) and occasionally beyond (central Scandinavia up to 64°). In North America wheat is grown to about 55° latitude. Wheat occurrence follows a similar pattern in the southern hemisphere. In the Alps, it is grown to an altitude of 1 500 metres above sea level (Geisler, 1991; Körber-Grohne, 1988).



Triticum aestivum
= Triticum vulgare

Fig. 86 Triticum aestivum: Illustrations from Ecopedia
http://fr.ekopedia.org/Image:Illustration_Triticum-aestivum.jpg#filelinks

2.1. Evolutionary history of wheat

(OECD, 1999): The oldest archaeological findings of naked wheat (6800 to 5200 B.C.) come from southern Turkey, Israel, Syria, Iraq, Iran and south of the Caucasus Mountains in Georgia. At that time, einkorn, emmer and barley were the staple cereal crops in Asia Minor. Wheat was only grown on a regional basis. There is evidence that naked wheat was cultivated in the southern Caucasus in neolithic settlements between the late fifth and early fourth millennium B.C. Late Bronze Age specimens (approximately 1000 to 900 B.C.) of naked wheat have been found at several sites in the Crimea, which was an early and significant wheat-growing area. Archaeological findings of wheat in Israel date from the same period (Körber-Grohne, 1988).

In Central Europe, the oldest dated findings of wheat grains (a mixture of *T. aestivum*, *T. dicoccon* and *T. monococcum*) were in soil samples from the New Stone Age (4600 to 3800 B.C.). When the late neolithic period began, naked wheat was gaining importance as a crop in some areas along the River Neckar and around riverside and moorland settlements in the northern foothills of the Alps. It was not until the Roman Empire that wheat spread to the lower Rhine regions, the lower Meuse and the Scheldt Estuary, where it became the main cereal crop. Further south, spelt was favoured. Wheat farming declined north of the Alps between the fall of Rome and the Middle Ages. Evidence from excavated sites shows that little wheat was grown in the period 800 to 1200 (Körber-

Grohne, 1988). The origin of Wheat has been well known since the 1940s, mainly through the work of E. R. Sears at the University of Missouri, Columbia (USA) from 1939 to 1980 (MacFadden & Sears, 1946) The evolution of wheat began with an unknown diploid prototype, from which the genera *Triticum* and *Aegilops* were formed by diploid divergence. The development of the genus *Triticum* (see Figure 2) began with the einkorn lineage (*T. monococcum* line, genome AA), which developed into the cultured form *T. monococcum* from the wild form *T. boeoticum*. Allopolyploidization with an *Ae. speltoides* descendant (genome BB) led to the tetraploid emmer lineage (*T. turgidum* line, genome AABB) with the wild form *T. dicoccoides* from which the cultured form *T. dicoccon* developed. The origin of the B-genome is more *uncertain; Ae. speltoides, Ae. longissimum, Ae. bicornis, Ae. searsii, Ae. sharonense are suggested as* possible progenitors. The spelt lineage[1] with the genome AABBDD resulted from further allopolyploidization with the species *Ae. squarrosa* (= *Ae. tauschii*; genome DD) (Misko & Germida, 2002), (Sitte et al., 1991), (Zeller & Friebe, 1991). For the current classification of the genus *Triticum* see the monograph of (Slageren van, 1994), also available on the home page of the Wheat Genetics Research Center, Kansas State University (http://www.ksu.edu/wgrc, under “Triticum” accessions). More recent references in regard to the issue of wheat origin are (Cauderon, 1994), (Zohary & Hopf, 1994) and (Feldman et al., 1995).

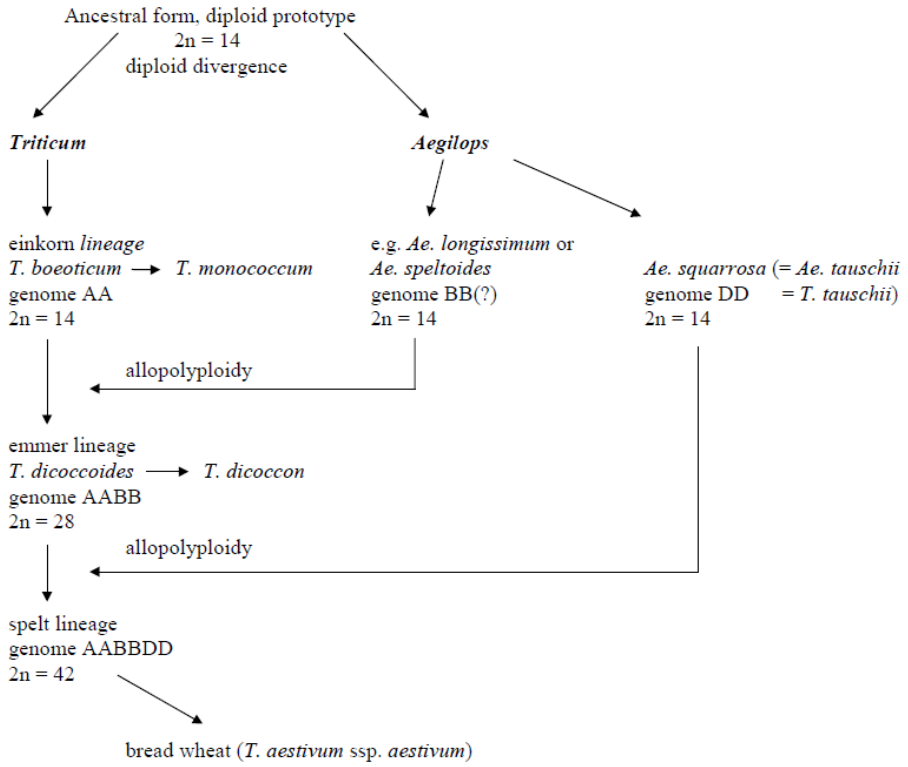


Fig. 87 Overview of the diploid Einkorn lineage (Körber-Grohne 1988, Sitte et al. 1991, Zeller und Friebe 1991), from (OECD, 1999)

2.1.1. Origin of Wheat and Natures Fields: Ancestral Monocultures

Botanists and plant collectors have according to (Wood & Lenne, 2001) repeatedly and emphatically noted the existence of dense stands of wild relatives of wheat. For example, in the Near East, (Harlan, 1992) noted that 'massive stands of wild wheats cover many square kilometers. (Hillmann, 1996) reported that wild einkorn (Triticum monococcum subsp. boeoticum) in particular tends to form dense stands, and when harvested its yields per square meter often match those of cultivated wheats under traditional management. (Harlan & Zohary, 1966) noted that wild Einkorn 'occurs in massive stands as high as 2000 meters [altitude] in south-eastern Turkey and Iran'. Wild emmer (Triticum turgidum subsp. dicoccoides) 'grows in massive stands in the northeast' of Israel, as an annual component of the steppe-like herbaceous vegetation and in the deciduous oak park forest belt of the Near East (Nevo, 1998). According to (Wood & Lenne, 2001) they are the strongest examples embracing wild progenitors of wheat: (Anderson, 1998) recorded wild wheat growing in Turkey and Syria in natural, rather pure stands with a density of 300/ m².

2.1.2. Origin of Einkorn lineage

The einkorn lineage includes the wild species of *T. boeoticum* and various goat grasses. The latter were formerly considered to belong to the genus *Aegilops,* but many geneticists now classify them as belonging to the genus *Triticum.* The only domesticated species in this group is einkorn *(T. monococcum). S*pecies have only one grain per floret; however, they may have one or two florets per spikelet. They are diploid (2n = 14, genome AA) (Körber-Grohne, 1988; Sitte et al., 1991; Zeller & Friebe, 1991).

2.1.3. Origin of Emmer lineage

The emmer lineage includes only tetraploid hybrids with the genome AABB. The cultivated form *T. dicoccon* developed from the wild form *T. dicoccoides*. Three forms of wild emmer are found today in various parts of Asia Minor and Central Asia. Of the six domesticated species, only emmer retains its hull as a mature grain. Species have two to three florets with two grains each (Körber-Grohne, 1988; Sitte et al., 1991; Zeller & Friebe, 1991).

2.1.4. Origin of Spelt lineage

It is assumed that genome A derives from einkorn (*T. monococcum*) and genome D from goat grass (*T. tauschii* = *Ae. squarrosa* = *Ae. tauschii*). The origin of the third genome (B) is still unclear. It possibly belongs to *Ae. speltoides* descendants or ancestors (see Section II: History of Wheat). The hexaploid wheat group (2n = 42, genome AABBDD) is closely related to spelt, macha and the naked wheats (see Table 3). The genetic differences in the gene pool of hexaploid wheat are small, although they exert a considerable influence, yielding both hulled grain (e.g. spelt) and naked grain (wheat).

The entire hexaploid lineage (AABBDD) is regarded as a single species. The various grains (e.g. bread wheat *T. aestivum* ssp. *vulgare*, spelt *Triticum aestivum* ssp. *spelta*) are considered as subspecies. In practical usage, however, the earlier categories are still frequently applied (Körber-Grohne, 1988).

2.2. Reproductive biology

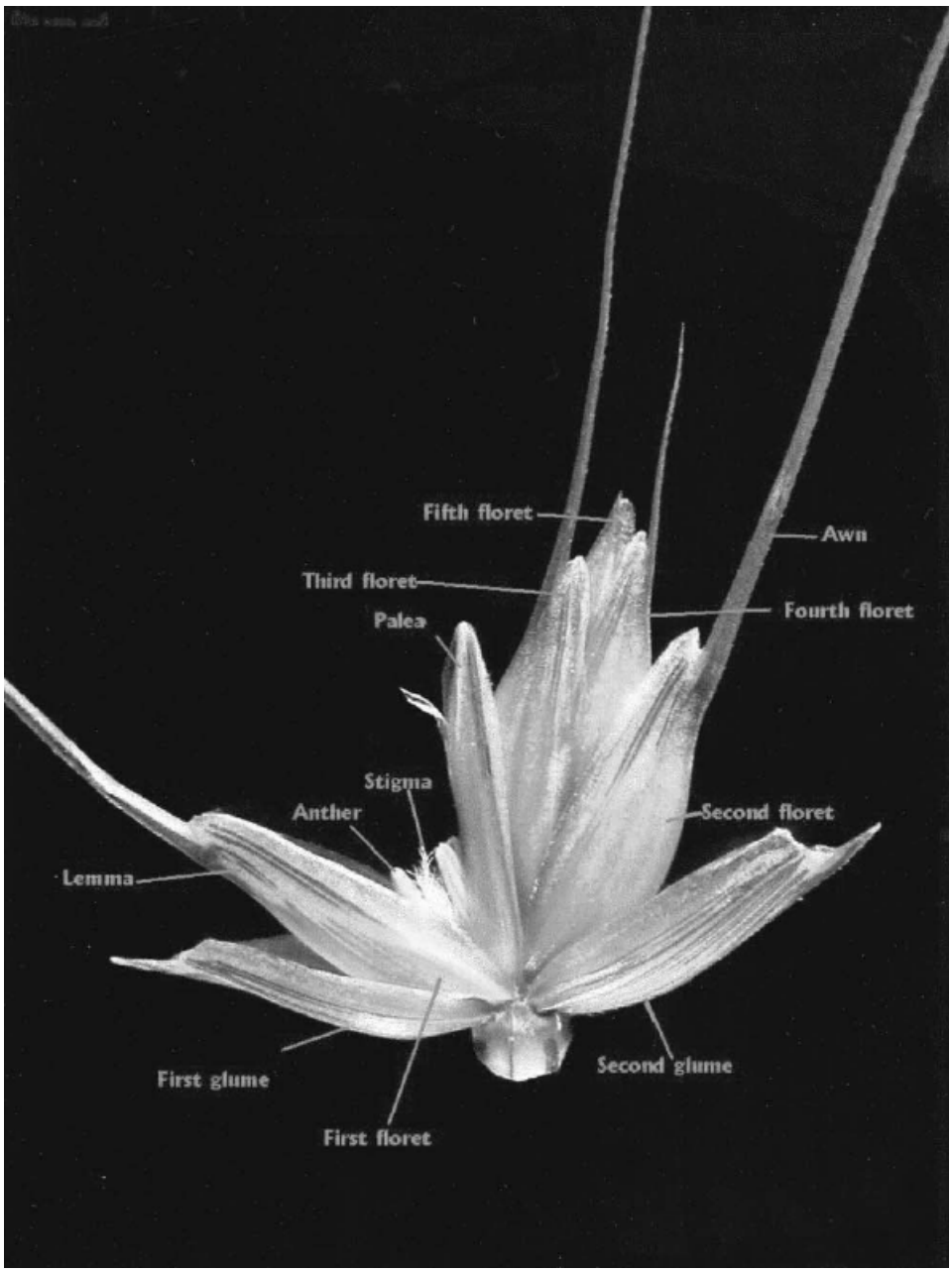


Fig. 88 Spikelet of wheat with five florets. The first floret on the left is open, showing the three anthers and a portion of the feathery stigma. [Courtesy: Research & Extension, Dep. of Agronomy, Kansas State Univ., Manhattan, KS; Available at: http://www.oznet.ksu.edu/pr_aawf/May/may_4.htm (verified 19 Sept. 2002).] From (Waines & Hegde, 2003)

Reproduction of *T. aestivum* is only known in the context of cultivation (Garcke, 1972).

Harvesting and propagation of its seed are entirely dependent on man. Wheat is predominantly selfpollinating.

The cross-fertilisation rate may be as high as 1 to 2 per cent, although it can be less than 1 per cent (Poehlmann, 1959). Wind-borne cross-fertilisation depends heavily on physical factors. It is minimal (0.1 per cent) where there is high humidity, but higher when there is warm, dry weather. Under such conditions, it has been claimed that the cross-fertilisation rate may be between 3.7 and 9.7 per cent.

Crossfertilisation is considerably more likely in the ears of stem branches (also called *tillers*) (Mandy, 1970). The rate of cross-fertilisation may also depend on the variety (e.g. Stoner 24 to 37 per cent). (Hucl, 1996) shows for 10 Canadian spring wheat cultivars that the cross-pollination frequency varies according to the genotype. The frequency was always lower than 9 per cent.

Apomixis is very rare (Mandy, 1970). Wheat's flowering season depends on geographical location. For example, in Germany and Sweden it flowers from late May to late June (Garcke, 1972; Mandy, 1970). Flowering times for Mediterranean Europe and the centres of origin and diversity of wheat are late winter, and early spring (Galun, personal communication). Sunny weather and temperatures of at least 11 to 13°C are propitious for flowering (Mandy, 1970). The influorescence of wheat is a spike, and the ear on the main culm flowers first. The process begins in the middle third of the ear, spreading towards the tip and base. The spikelets at the top and bottom of the ear are the last to bloom (Mandy, 1970). In cultivated wheat fields, the number of ears is usually between 400 and 650/m². Depending on the proportion of well-developed ears, the average grain count per ear varies between 35 to 40 and 20 to 25. However, the standard number of seeds per head is 30 to 35 (one ear carrying an average of 80 florets) (Kübler, 1994); average data in Germany). When flowering, the lemmas and palaeas open to an angle of 20 to 35°. The pollen sacs appear about four to six minutes later adopting a horizontal position. Under favourable weather conditions a floret will complete the flowering cycle in 13 to 18 minutes. The reproductive organs are slightly protandrous (pollen sacs mature one to three days earlier). An unfertilised spikelet remains open for several hours or even days (Mandy, 1970).

Flowering for a full ear takes between 101 and 120 hours, 23 florets a day blooming on average. Blooming begins in the early morning between 4 and 5 a.m. Peak flowering time is between 9 and 10 a.m., with a second peak between 2:30 and 3:30 p.m. By 7 p.m. flowering is usually completed. A wheat plant flowers for four to 15 days (Mandy, 1970); average data in Germany). The quantity of pollen produced by an anther is low, being approximately 2700 pollen grains per sac. It has been established that, on average, 80 per cent of pollen from an anther which protrudes from the spikelet is dispersed into the air. It was assumed from this that a wheat variety with a large number of protruding anthers would make enough pollen available to achieve cross-fertilisation. Under experimental conditions in the laboratory (moderate mass exchange of 10 g/cm per second and moderate wind speed of 3 m/sec), pollen travels about 60 m distance at a height of 1 m (D'Souza, 1970). In field experiments (Wilson, 1968) found 10 per cent seedsetting on male sterile wheat plants that were 30 m from the pollen donor plants.

Pollen begins to germinate 15 minutes after deposition on the stigma (D'Souza 1970) and retains its fertilisation ability for only a very short period. Even under optimum conditions of 5°C and 60 per cent relative atmospheric humidity, this period will not exceed three hours. Under common field conditions of 20°C and 60 per cent relative atmospheric humidity it may remain viable for less than 30 minutes. With temperatures of about 30°C and low relative atmospheric humidity, the pollen is only able to achieve its function for 15 minutes. On hot days, therefore, this short fertilisation period can considerably reduce pollen germination in the event that cross-pollination does occur (D'Souza, 1970).

3. Biosafety considerations

3.1. Summary of gene flow in wheat cultivars, derived from realistic field experiments

A comprehensive summary of gene flow experiments has been given by (Gustafson et al., 2005) in a review of pollen mediated gene flow (PMGF) from a series of papers on gene flow from the literature. They carefully eliminated studies done with male sterile receptor plants and fields, since they are not representative with their high values of gene flow.

Table 1. Levels of pollen-mediated gene flow (PMGF) reported in wheat by various researchers.

Study	Pollinator size	Distance (range of distances) where PMGF was measured	Mean PMGF observed
		m	%
Harrington (1932)	5-m row. Female parents and pollinators planted in tandem pairs.	0.3	0.88
Griffin (1987)	3.6-m row. 3-row double plots.	0.6	1.11
Martin (1990)	6-m row. Two rows of pollinator on either side.	0.3	1.20
Hucl (1996)	6-m row. Two rows of pollinator on either side.	0.25	0.89
Hucl and Matus-Cadiz (2001)	5- × 5-m block	(0–33)	0.15
Matus-Cadiz et al. (2004)	50- × 50-m block	(0–160)	0.04 (wheat/wheat)
		(0–260)	0.01 (wheat/durum)

Fig. 89 Levels of pollen-mediated gene flow (PMGF) roported in wheat by various reserarch groups, from (Gustafson et al., 2005)

The extent of PMGF in wheat is low, largely because wheat is predominantly (>99%) self-pollinating (Harrington, 1932). The floral biology of wheat is such that stigma maturation for pollen receptivity and pollen shed from mature anthers are in phase within a floret. This means that, in each discrete floret, stigmas are usually pollinated by pollen shed from anthers located in the same floret. However, it is recognized that low levels of cross-pollination can occur in wheat. The following observations have been made regarding cross-pollination in wheat:

- The amount of gene flow that occurs is usually low (typically below 1%) even for plants in close proximity (Chamberlain & Stewart, 1999; De Vries, 1971; De Vries, 1974a, b; Jensen, 1968; Lelley, 1966)
- The amount of gene flow decreases with greater distance between the pollen donor and recipient plant (D'Souza, 1970; De Vries, 1971; Hucl, 1996; Jensen, 1968; Khan et al., 1973; Suneson & Cox, 1964; Virmani & Edwards, 1983)

- There are genotypic differences for flowering traits among wheat cultivars and this is due to deliberate selec-tion by plant breeders (Allan, 1980; Harrington, 1932; Hucl, 1996; Hucl & Matus-Cadiz, 2001; Waines & Hegde, 2003). Wheat genotype affects the number of anthers per spikelet (D'Souza, 1970; De Vries, 1971; Joppa et al., 1968), the extent of flower opening and duration of flowering (Nonaka et al., 1993; Tsunewaki, 1969, 1993), anther size and stigma size (Cahn, 1925; De Vries, 1974b; Kherde et al., 1967), and the amount of pollen produc-tion (Anand & Beri, 1971; Beri & Anand, 1971; Cahn, 1925; D'Souza, 1970; Joppa et al., 1968; Khan et al., 1973; Pohl, 1937)
- Wheat pollen is viable for a relatively short period of time (typically 30–60 minutes) (D'Souza, 1970; De Vries, 1971)
- Wheat produces a relatively small amount of pollen (D'Souza, 1970; Pohl, 1937)
- Wheat pollen is relatively heavy compared with pollen of other grass species (Lelley, 1966) causing it to settle quickly. Wind is required to move wheat pollen an appreciable distance from the source (D'Souza, 1970; Dowding, 1987; Pickett & Galwey, 1997; Waines & Hegde, 2003; Zuzens et al., 1969)
- No purposeful insect vectors are known (Eastham & Sweet, 2002; OECD, 1999). Pollinating insects such as bees are infre-quent visitors due to low pollen production and lack of nectaries in the wheat flower (De Vries, 1971)
- Environmental factors such as temperature and humid-ity affect wheat pollen viability, mobility, and male sterility within the receptor, with high humidity making pollen heavier and high temperatures reducing pollen viability (Beri & Anand, 1971; Bitzer & Patterso.Fl, 1967; De Vries, 1971; Jensen, 1968; Leighty & Sandok, 1924; Livers, 1964). Under drought conditions, unfertilized florets remain open, exposing the stigma for 2 to 3 d and shedding pollen into the air (Hoshikawa, 1960; Laurie et al., 2004; Molnar-Lang et al., 1980; Sadras & Monzon, 2006; Yang et al., 2006)
- Environmental factors also affect the percentage of extruding anthers in the wheat spikelet, duration of flower opening and pollen shed, and stigma receptivity (D'Souza, 1970; De Vries, 1971; Khan et al., 1973; Khan et al., 1971; Livers, 1964; Major, 1980). Under stressed conditions, 30 to 80% of the pollen is shed outside the wheat flower (Beri & Anand, 1971; D'Souza, 1970; Leighty & Sandok, 1924). Heat reduces both the duration of pollen shed and stigma receptivity to pollen (D'Souza, 1970; De Vries, 1971; Heslop-Harrison, 1979; Major, 1980). See more recent literature about the subject: (Ghaemi et al., 1993; Lu et al., 1991; Orshinsky & Sadasivaiah, 1997; Pauk et al., 1991; Subedi et al., 1998; Westgate et al., 1996; Xynias et al., 2001)

Summarizing the net impact of environmental factors, maximum levels of PMGF in wheat probably occur when a hot, dry period is followed by a period of moderate temperatures with high humidity and wind. This allows for maximum pollen viability, flower opening, and stigma receptivity as well as pollen dispersal.

3.2. Modelling efforts of gene flow from wheat to wheat

Following again (Gustafson et al., 2005): Most of the previous work on modeling PMGF in crops has focused on mechanistic approaches. In general, this has involved the modification of air pollution models based on the Gaussian plume equation, to simulate pollen transport from source to receptor fields. Theoretical attempts to directly model the complex fluid mechanics of air flow across, between, and within plant canopies may be broadly categorized as either Eulerian or Lagrangian (Di-Giovanni et al., 1989; Di-Giovanni & Kevan, 1991). The former class of models uses a fixed set of points in space as the control volume, whereas the latter considers a control volume of fluid as it moves through space. Advantages for the Lagrangian system in the case of pollen flow have been claimed (Rodean, 1995), but both theoretical systems have significant challenges in addressing the varied but specific crop reproductive biology characteristics and the topographic variations of the agricultural landscape. A further challenge to the mechanistic approach is that PMGF involves several additional steps beyond predicting how far the pollen moves and in what relative concentrations (Aylor & Ferrandino, 1989; Aylor et al., 1993; Leclerc et al., 1988; Legg & Powell, 1979; Raynor et al., 1972; Raynor et al., 1971). In order for successful gene flow to occur, the pollen must land on the stigma of the receptor plant, stick to the surface, germinate, and fertilize the ovule. In addition, it must compete with other native and foreign pollen that may land on the stigmatic surface. Validated mechanistic models of these processes are currently not available and may be difficult to develop without considerable empiricism.

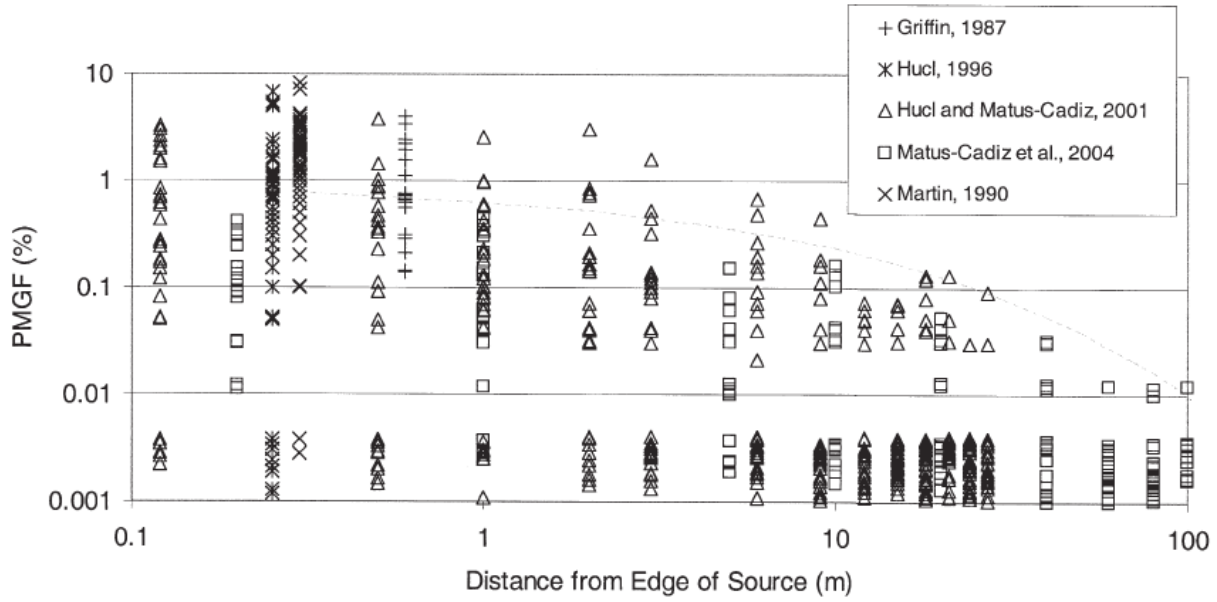


Fig. 90 Comparison of the General Wheat Model to all available individual PMGF observations in the referenced field studies, showing its conservative ("high-end") nature. Jittering (see text) around the PMGF detection limit of each study has been used to display all individual sample points on the plot. The model appears as a curve due to the use of a logarithmic scale for the *x* axis. From (Gustafson et al., 2005)

The future may bring combination models, such as those hybrid models already put in place for prediction of pollution in coastal systems: (Suh, 2006). Another challenge is to adapt the models to complex landscape structures (Palau et al., 2006). Overall, gene flow in wheat is relatively low, since most wheat cultivars are strong inbreeders.

3.3. Wheat to Wheat gene flow: Application of the empirical model

3.3.1. Scale Effect

According to (Gustafson et al., 2005) the empirical model was used to simulate the effect of source size on PMGF. The authors made two key assumptions in carrying out such calculations.

1. The model (which was fit to data for a 50-m source, with non-zero observations out to only 100 m) continues to remain valid for longer distances.
2. The PMGF produced by adjacent sources can simply be added together in a linear fashion to predict the effect of increasing source size and therefore increased pollen load. This assumption is likely valid for the rather low PMGF observed in wheat, but would obviously break down for higher gene flow percentages.

With these two assumptions, it was possible to investigate the predicted effect of source size by simply adding together the predicted gene flow from multiple adjacent sources. An example of this is shown in Fig. 6 for the GWM. The model appears as a concave-upward curve due to the use of the linear scale for the x axis. As the width of the source increases from 50 to 800 m (corre-sponding to square field sizes of 0.25 to 64 ha), only a minimal effect on PMGF is predicted. The 400 m (16 ha) and 800 m (64 ha) curves are practically indistinguish-able, suggesting that asymptotic PMGF is attained for sources greater than about 10 ha. The fact that scaling effects are predicted to be so small is logically consistent with the empirical field observation that PMGF pro-duced at a distance equal to the width of the pollinator source is near zero. Thus, the incremental PMGF arising from increasing the source width is negligible.

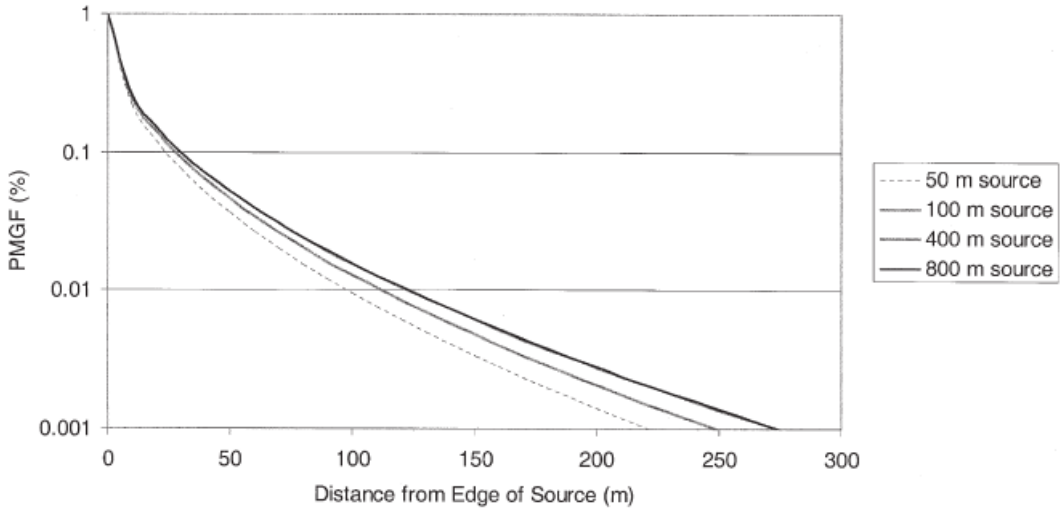


Fig. 91 Use of the General Wheat Model to study the effect of the size of the pollinator source. The model appears as a curve due to the use of the linear scale for the *x* axis. From (Gustafson et al., 2005)

The assumption of the validity of the model for longer distances is contested by (Willenborg & Van Acker, 2006) with the following arguments:

They considered the model used problematic because long distance PMGF (the tail of the gene flow curve) is the most important aspect of gene flow due to its effects on meta-population structure and plant population dynamics (Austerlitz et al., 2004). (Willenborg & Van Acker, 2006) find it, among other critical points, concerning that no consideration was given to describing the tail of the gene flow curve in the manuscript given that the most fundamental task in studying dispersal is describing the dispersal pattern.

(Gustafson et al., 2006) reply that many arguments of (Willenborg & Van Acker, 2006) stem from research with other crops such as oilseed rape, which they do not consider to be relevant for their case and in addition they claim that the wheat data do not contain a considerable 'tail of the gene flow curve'. Empirical data of wheat breeders would fit into the view of (Gustafson et al., 2005).

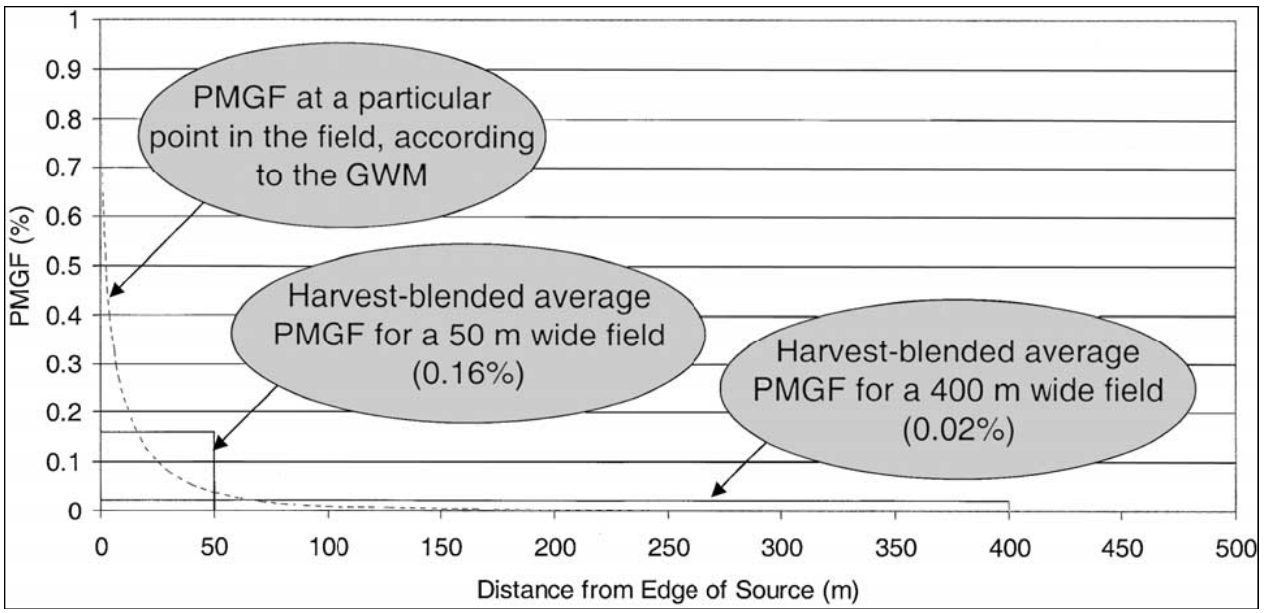


Fig. 92 Use of the General Wheat Model to demonstrate the impact of harvest-blending on PMGF. From (Gustafson et al., 2005)

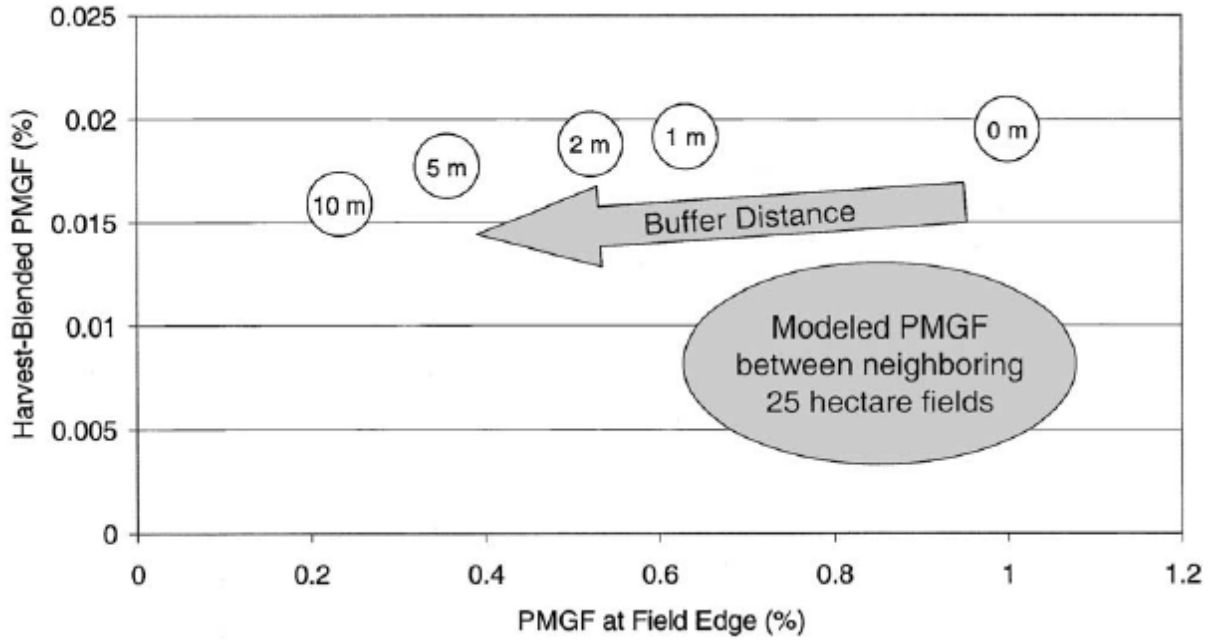


Fig. 93 Use of the General Wheat Model to study the influence of buffer distance on PMGF. From (Gustafson et al., 2005)

As shown in Fig. 8, the impact of blending at harvest is predicted to result in dramatic dilution of PMGF at the field level. The x axis indicates the PMGF predicted by the GWM at the edge of the field directly adjacent to the source, whereas the y axis shows the

corresponding harvest-blended PMGF across an entire 25-ha field, for various isolation buffer widths up to 10 m (see below). The results show that the harvest-blended PMGF is predicted to be extremely low regardless of buffer width. The ratio of harvest-blended PMGF to field-edge PMGF shows a reduction factor of about 10 to 50, depending on isolation buffer width. Isolation distances reduce the amount of gene flow to the nearest edge of the receptor field, but are predicted to have no measurable impact on harvest-blended PMGF for these neighboring 25-ha fields because the dilution effect dominates at the field scale.

In a last figure 9, (Gustafson et al., 2005) demonstrate the influence of the receptor field width for a variety of buffer distances.

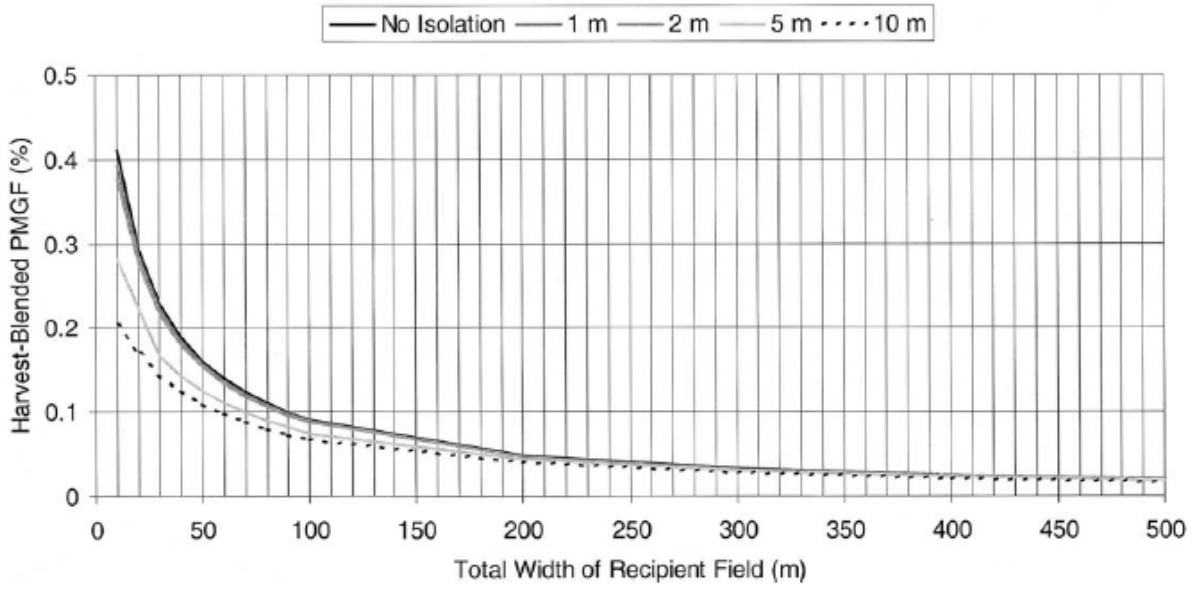


Fig. 94 Use of the General Wheat Model to study the influence of receptor field width for a variety of buffer distances. From (Gustafson et al., 2005).

3.4. Summary of Gene Flow from Wheat to wild relatives

Wheat is a self-compatible, wind-pollinated species whose flowers are often cleistogamous. Crop-to-crop gene flow is very limited. Field experiments demonstrate that the distance of wheat pollen dispersal resulting in hybrids is usually a few meters. The safe distance does not seem to exceed 1 meter to keep intermixing of traits below 0.5%. According to a summary in (Jacot et al., 2004) crop-to-wild gene flow have been detected between wheat and *Aegilops ovata* in the field or in herbarium specimens. Back-crosses and F1 and F2 progenies are known from experimental gardens. However, there is no evidence that hybrids are stabilized in any of the populations observed. Spontaneous amphiploidy could be the main avenue for transferring genes from cultivated durum wheat to wild *Aegilops ovata*. Artificial hybridization with wheat used as pollen donor can reach higher levels for some species like *Aegilops cylindrica*, *Aegilops ovata* or *Aegilops biuncialis*, but is generally very low with other wild relatives. Risk assessment of gene escape from wheat should base on these hybridization limits. Artificial hybridization rates have been studied by means of cultivation experiments in the greenhouse (Loureiro et al., 2001; Lu et al., 2002; Zhao et al., 2000). Fourteen species were studied. Bread wheat has been crossed as pollinator with *Agropyron repens*, *Aegilops bicornis*, *Aegilops biuncialis, Aegilops cylindrica, Aegilops neglecta*, *Aegilops ovata*, *Aegilops peregrina*, *Aegilops speltoides, Aegilops squarrosa, Aegilops tauschii*, *Aegilops triuncialis*, *Elytrigia elongata, Roegneria ciliaria, Secale cereale*, *Triticum durum, T. monococcum*, *T. timopheevii* ssp*. armeniacum* and *T. turgidum* ssp*. dicoccoides. Aegilops neglecta, Aegilops ovata* and *Aegilops triuncialis* were also crossed with durum wheat*.*

In a first set of experiments, several individuals of wild relatives were emasculated (glumes not cut). The first third was hand pollinated with wheat pollen. The green seeds formed were collected and their embryos were cultivated in vitro. The second third was hand pollinated and seeds were maintained on the plants until maturation. The last third was surrounded by flowering plants of wheat and seeds were collected at maturity. Those experiments were repeated two times, in 2000 and 2001.
In a second series of experiments, wheat relatives were castrated before flowering and artificially pollinated twice in the following 1-2 days with mixed pollen of *Triticum aestivum*. One drop (10 □l) of GA_3 (50 mg/l) or 2.4-D (100 mg/l) was applied to each castrated floret during the second and third day after pollination. Twelve to seventeen days later, the hybrid spikes were collected and the rate of seed-set was determined.

Artificial hybridization between wheat used as a pollen donor and wild relatives is likely to occur at low rates. After hand pollination, hybrids were observed between wheat and 12 out of 17 related taxa. Pollen of *Triticum durum* was able to sire viable seeds with all three *Aegilops* species present in the experiment*.* Hybrids appear to have intermediate characters but most of them are at least partially sterile.

In a thesis of the University of Neuchatel (Schönenberger, 2005) show that wheat pollen that may compete with *Ae.cylindrica* pollen to fertilise its ovules does not fly very far from

a field. In fact, we detected one single hybridisation event at 1 m from a wheat field's edge, representing a frequency of 0.29% in that plot. No hybridisation was detected at greater distances, neither at 1 m in field 2. When considering a 95% confidence interval, hybridisation rates are always below 0.9% for the distances greater then 5 meters. The presence of cultivated oat between pollen donor fields and the *Ae. cylindrica* plots probably reduced hybridisation distances, although the *Ae. cylindrica* spikes had the same height as the oat canopy. This corresponds to natural situations, where *Ae. cylindrica* grows mixed with other grasses and dicotyledons. Although based on a limited sample size, we can conclude with a certain confidence that minimum isolation distances to effectively avoid hybridisation are small, of the order of a few meters, see also the more detailed studies (Schoenenberger et al., 2005; Schoenenberger et al., 2006) and (Guadagnuolo et al., 2001a; Guadagnuolo et al., 2001b).

Triticum, Wheat risk assessment scheme

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) southern Europe yes, northern Europe no**	**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)**	**1-(2)-((3))**
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)**	**2**
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, very low**
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)**	**1-(2)-((3))**
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)**	**0**
CPC4	**If fertilization happens, will a viable F_1 individual be established itself from hybrid seed in absence of mechanical/chemical control? (0/1)**	**0 ((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)**	**0 ((1))**

CSF	**Propensity for successful seed- and tiller mediated gene flow from commercial crop to feral**	
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)**	**0 (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)**	**3 (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**

7. Cited literature

Abe, J., Yoshikawa, H., & Tsuda, C. (1986)
Reproductive barriers in sugar beet and its wild relatives of the section Vulgares, the genus Beta. J Fac Agric Hokkaido Univ, 63, pp 40-48

Abrahamson, L.P., White, E.H., Nowak, C.A., Briggs, R.D., & Robison, D.J. (1990)
Evaluating Hybrid Poplar Clonal Growth-Potential in a 3-Year-Old Genetic Selection Field Trial. Biomass, 21, 2, pp 101-114
<Go to ISI>://A1990CM23300002

Adati, S. & Shiotani, I. (1962)
The Cytotaxonomy of the genus Miscanthus and its phylogenetic status. Bull. Fac. Agr. Mic. Univ., 25, pp 1-24

Al-Ahmad, H., Dwyer, J., Moloney, M., & Gressel, J. (2006a)
Mitigation of establishment of Brassica napus transgenes in volunteers using a tandem construct containing a selectively unfit gene. Plant Biotechnology Journal, 4, 1, pp 7-21
<Go to ISI>://000234030000003 AND http://www.botanischergarten.ch/Geneflow/AlAchmad-Tandem-napus-2006.pdf

Al-Ahmad, H., Galili, S., & Gressel, J. (2004)
Tandem constructs to mitigate transgene persistence: tobacco as a model. Molecular Ecology, 13, 3, pp 697-710
<Go to ISI>://000188825700016 AND http://www.botanischergarten.ch/Geneflow/Al-Ahmad-Gressel-Tandem-2004.pdf

Al-Ahmad, H., Galili, S., & Gressel, J. (2006b)
Infertile interspecific hybrids between transgenically mitigated Nicotiana tabacum and Nicotiana sylvestris did not backcross to N. sylvestris. Plant Science, 170, 5, pp 953-961
<Go to ISI>://000236517700006 AND http://www.botanischergarten.ch/Geneflow/AlAchmad-Nobackcross-2006.pdf

Al-Ahmad, H. & Gressel, J. (2005)
Transgene containment using cytokinin-reversible male sterility in constitutive, gibberellic acid-insensitive (Delta gai) transgenic tobacco. Journal of Plant Growth Regulation, 24, 1, pp 19-27
<Go to ISI>://000231700700004 AND http://www.botanischergarten.ch/Geneflow/AlAchmad-Cytokinin-2005.pdf

Al-Ahmad, H. & Gressel, J. (2006)
Mitigation using a tandem construct containing a selectively unfit gene precludes establishment of Brassica napus transgenes in hybrids and backcrosses with weedy Brassica rapa. Plant Biotechnology Journal, 4, 1, pp 23-33
<Go to ISI>://000234030000004 AND http://www.botanischergarten.ch/Geneflow/AlAchmad-Tandem-Establishment-2006.pdf

Al-Janabi, S.M., McClelland, M., Petersen, C., & Sobral, B.W.S. (1994)
Phylogenetic analysis of organellar DNA sequences in the Andropogoneae: Saccharinae. TAG Theoretical and Applied Genetics, 88, 8, pp 933-944
http://dx.doi.org/10.1007/BF00220799 AND http://www.botanischergarten.ch/Africa-Harvest-Sorghum-Lit-1/Al-Janabi-Andropogoneae-1994.pdf

Al-Shehbaz, I.A., Beilstein, M.A., & Kellogg, E.A. (2006)
Systematics and phylogeny of the Brassicaceae (Cruciferae): an overview. Plant Systematics and Evolution, 259, 2-4, pp 89-120
<Go to ISI>://000238998800003 AND http://www.botanischergarten.ch/EPOBIO-Brassica/Al-Shebaz-Systematics-2006.pdf

Alemayehu, N. & Becker, H. (2002)
Genotypic diversity and patterns of variation in a germplasm material of Ethiopian mustard (Brassica carinata A. Braun). Genetic Resources and Crop Evolution, 49, 6, pp 573-582
<Go to ISI>://000179520000006

Allainguillaume, J., Alexander, M., Bullock, J.M., Saunders, M., Allender, C.J., King, G., Ford, C.S., & Wilkinson, M.J. (2006)

Fitness of hybrids between rapeseed (Brassica napus) and wild Brassica rapa in natural habitats. Molecular Ecology, 15, 4, pp 1175-1184
<Go to ISI>://000235986700023 AND http://www.botanischergarten.ch/EPOBIO-Brassica/Allainguillaume-Fitness-2006.pdf

Allan, R.E. (1980)
Wheat. *In Hybridization of crop plants* (eds W.R. Fehr & H.H. Hadley), pp. 709-720. ASA and CSSA, Madison, WI

Alliende, M.C. & Harper, J.L. (1989)
Demographic-Studies of a Dioecious Tree .1. Colonization, Sex and Age Structure of a Population of Salix-Cinerea. Journal of Ecology, 77, 4, pp 1029-1047
<Go to ISI>://A1989CF85700009 AND http://www.botanischergarten.ch/EPOBIO-Salix/Alliende-Demographic-1989.pdf

Alshehbaz, I.A. (1985)
The Genera of Brassiceae (Cruciferae, Brassicaceae) in the Southeastern United-States. Journal of the Arnold Arboretum, 66, 3, pp 279-351
<Go to ISI>://A1985AMA6600001

Amalraj, V. & Balasundaram, N. (2005)
On the Taxonomy of the Members of 'Saccharum Complex'. Genetic Resources and Crop Evolution, V53, 1, pp 35-41 http://dx.doi.org/10.1007/s10722-004-0581-1 AND http://www.botanischergarten.ch/EPOBIO-Miscanthus/Amalraj-Saccharum-Complex-2005.pdf

Ammann, K. & Jacot, Y. (2003)
Vertical Gene Flow. *In Methods for Risk Assessment of Transgenic Plants* (eds K. Ammann, Jacot, Y. & R. Braun). Birkhäuser, Basel

Ammann, K., Jacot, Y., Kjellson, G., & Simonsen, V., eds. (1999)
III. Ecological risks and prospects of transgenic plants, where do we go from here? A dialogue between biotech industry and science, Vol. III, pp 260, Methods for Risk Assessment of Transgenic Plants III Birkhauser, Basel, IS: 3-7643-5917-X
http://www.springer.com/chl/home/default?SGWID=2-40356-22-2029826-0

Ammann, K., Jacot, Y., & Rufener Al Mazyad, P. (1996)
Field release of transgenic crops in Switzerland : an ecological assessment of vertical gene flow. *In Gentechnisch veränderte krankheits- und schädlingsresistente Nutzpflanzen. Eine Option für die Landwirtschaft ?* (eds E. Schulte & O. Käppeli), Vol. 1, 3, pp. 101-157. Schwerpunktprogramm Biotechnologie, Schweiz. Nationalfonds zur Förderung der Wissenschaftlichen Forschung, BATS, Basel http://www.botanischergarten.ch/debate/techdef5a.pdf

Ammann, K., Jacot, Y., & Rufener Al Mazyad, P. (2000)
an Ecological Risk Assessment of Vertical Gene Flow. *In Safety of Genetically Engineered Crops* (ed R. Custers). Flanders Interuniversity Institute for Biotechnology, Zwijinarde, BE.J. Bury, VIB VIB Publication
http://www.vib.be and http://www.botanischergarten.ch/Geneflow/VIBreport.pdf

Ammann, K., Jacot, Y., & Rufener Al Mazyad, P. (2003)
An Ecological Risk Assessment of Vertical Gene Flow. *In Methods for Risk Assessment of Transgenic Plants IV* (eds K. Ammann, R. Braun & Y. Jacot), pp. 19-35. Birkhauser, Basel The link to the whole book:
http://www.springer.com/chl/home/birkhauser/biosciences?SGWID=2-40293-22-2200692-0 AND
http://www.botanischergarten.ch/Geneflow/Ammann-verticalGeneFlow3.pdf

Ammann, K. & Papazova Ammann, B. (2004)
Factors Influencing Public Policy Development in Agricultural Biotechnology. *In RISK ASSESSMENT OF TRANSGENIC CROPS.* (ed S. Shantaram), Vol. 9, pp. 1552. Wiley and Sons, Hoboken, NJ, USA.P. Christou & H. Klee Handbook of Plant Biotechnology
http://www.botanischergarten.ch/Wiley/Factors-Discourse-Wiley.pdf

Anand, S.C. & Beri, S.M. (1971)
Seed Setting in Male Sterile Wheat. Indian Journal of Genetics and Plant Breeding, 31, 1, pp 132-&
<Go to ISI>://A1971K676200022

Anderson, P.C. (1998)
History of harvesting and threshing techniques for cereals in the prehistoric Near East. *In The Origins of Agriculture and Crop Domestication* (eds A.B. Damania, J. Valkoun, G. Willcox & C.O. Qualset), pp. 145-159. ICARDA, Aleppo

Andersson, N.J. (1856)
Miscanthus. Oefv. Svensk. Vet. Akad. Forh. Stock., 7, pp 165

Archimowitsch, A. (1949)
Control of Pollination in Sugar-Beet. Botanical Review, 15, 9, pp 613-628
<Go to ISI>://A1949XS42800002 AND NEBIS

Arnaud, J.F., Viard, F., Delescluse, M., & Cuguen, J. (2003)

Evidence for gene flow via seed dispersal from crop to wild relatives in Beta vulgaris (Chenopodiaceae): consequences for the release of genetically modified crop species with weedy lineages. Proceedings of the Royal Society of London Series B-Biological Sciences, 270, 1524, pp 1565-1571
<Go to ISI>://000184689100004 AND http://www.botanischergarten.ch/EPOBIO-Beta/Arnaud-Evidence-seed-2003.pdf

Arnoldo, M., Baszczynski, C.L., Bellemare, G., Brown, G., Carlson, J., Gillespie, B., Huang, B., Maclean, N., Macrae, W.D., Rayner, G., Rozakis, S., Westecott, M., & Kemble, R.J. (1992)
Evaluation of Transgenic Canola Plants under Field Conditions. Genome, 35, 1, pp 58-63
<Go to ISI>://A1992HJ35100010

Austerlitz, F., Dick, C.W., Dutech, C., Klein, E.K., Oddou-Muratorio, S., Smouse, P.E., & Sork, V.L. (2004)
Using genetic markers to estimate the pollen dispersal curve. Molecular Ecology, 13, 4 %R doi:10.1111/j.1365-294X.2004.02100.x, pp 937-954
http://www.blackwell-synergy.com/doi/abs/10.1111/j.1365-294X.2004.02100.x AND
http://www.botanischergarten.ch/EPOBIO-Wheat/Austerlitz-Markers-2004.pdf

Aylor, D.E. & Ferrandino, F.J. (1989)
Dispersion of Spores Released from an Elevated Line Source within a Wheat Canopy. Boundary-Layer Meteorology, 46, 3, pp 251-273
<Go to ISI>://A1989T604500003

Aylor, D.E., Wang, Y.S., & Miller, D.R. (1993)
Intermittent Wind Close to the Ground within a Grass Canopy. Boundary-Layer Meteorology, 66, 4, pp 427-448
<Go to ISI>://A1993MJ07300005

Azuma, T., Kajita, T., Yokoyama, J., & Ohashi, H. (2000)
Phylogenetic relationships of Salix (Salicaceae) based on rbcL sequence data. Am. J. Bot., 87, 1, pp 67-75
http://www.amjbot.org/cgi/content/abstract/87/1/67 AND http://www.botanischergarten.ch/EPOBIO-Salix/Azuma-Phylogenetic-2000.pdf

Baillargeon, G. (1986)
Taxonomische Revision der Gattung Sinapis (Cruciferae, Brassicaceae), FU Berlin, Berlin Thesis, pp

Baranski, R., Grzebelus, D., & Frese, L. (2001)
Estimation of genetic diversity in a collection of the Garden Beet Group. Euphytica, 122, 1, pp 19-29
<Go to ISI>://000172070100003 AND http://www.botanischergarten.ch/EPOBIO-Beta/Baranski-Estimation-2001.pdf

Barker, N.P., Clark, L.G., Davis, J.I., Duvall, M.R., Guala, G.F., Hsiao, C., Kellogg, E.A., Linder, H.P., Mason-Gamer, R.J., Mathews, S.Y., Simmons, M.P., Soreng, R.J., & Spangler, R.E. (2001)
Phylogeny and subfamilial classification of the grasses (Poaceae). Annals of the Missouri Botanical Garden, 88, 3, pp 373-457
<Go to ISI>://000171142100001

Barnes, B.V. (1966)
Clonal Growth Habit of American Aspens. Ecology, 47, 3, pp 439-&
<Go to ISI>://A19668082200009

Barnes, B.V. (1975)
Phenotypic Variation of Trembling Aspen in Western North-America. Forest Science, 21, 3, pp 319-328
<Go to ISI>://A1975AV99100020

Barrett, J.W., Rajora, O.P., Yeh, F.C.H., Dancik, B.P., & Strobeck, C. (1993)
Mitochondrial-DNA Variation and Genetic-Relationships of Populus Species. Genome, 36, 1, pp 87-93
<Go to ISI>://A1993KP62300012

Barsoum, N. (2001)
Relative contributions of sexual and asexual regeneration strategies in Populus nigra and Salix alba during the first years of establishment on a braided gravel bed river. Evolutionary Ecology, 15, 4-6, pp 255-279
<Go to ISI>://000176502000003 AND http://www.botanischergarten.ch/EPOBIO/Barsoum-Asexual-2001.pdf

Barsoum, N., Muller, E., & Skot, L. (2004)
Variations in levels of clonality among Populus nigra L. stands of different ages. Evolutionary Ecology, 18, 5-6, pp 601-624
<Go to ISI>://000229506200011 AND http://www.botanischergarten.ch/EPOBIO/Barsoum-Clonality-2004.pdf

Bartsch, D., Brand, U., Morak, C., Pohl-Orf, M., Schuphan, I., & Ellstrand, N.C. (2001)
Biosafety of hybrids between transgenic virus-resistant sugar beet and Swiss chard. Ecological Applications, 11, 1, pp 142-147
<Go to ISI>://000166749100012 AND http://www.botanischergarten.ch/EPOBIO-Beta/Bartsch-Hybrids-2001.pdf

Bartsch, D. & Ellstrand, N.C. (1999)

Genetic evidence for the origin of Californian wild beets (genus Beta). Theoretical and Applied Genetics, 99, 7-8, pp 1120-1130
<Go to ISI>://000084014900004 AND http://www.botanischergarten.ch/EPOBIO-Beta/Bartsch-Evidence-1999.pdf

Bartsch, D., Lehnen, M., Clegg, J., Pohl-Orf, M., Schuphan, I., & Ellstrand, N.C. (1999)
Impact of gene flow from cultivated beet on genetic diversity of wild sea beet populations. Molecular Ecology, 8, 10, pp 1733-1741
<Go to ISI>://000083466800016 http://www.botanischergarten.ch/EPOBIO-Beta/Bartsch-Impact-1999.pdf

Bartsch, D. & Schmidt, M. (1997)
Influence of sugar beet breeding on populations of Beta vulgaris ssp. maritima in Italy. Journal of Vegetation Science, 8, 1, pp 81-84
<Go to ISI>://A1997XC68800011

Bateman, A.J. (1947a)
Contamination of Seed Crops .1. Insect Pollination. Journal of Genetics, 48, 2, pp 257-275
<Go to ISI>://A1947XX16800012

Bateman, A.J. (1947b)
Contamination of Seed Crops .2. Wind Pollination. Heredity, 1, 2, pp 235-246
<Go to ISI>://A1947YB41500005

Beaudoin, M., Hernandez, R.E., Koubaa, A., & Poliquin, J. (1992)
Interclonal, Intraclonal and within-Tree Variation in Wood Density of Polar Hybrid Clones. Wood and Fiber Science, 24, 2, pp 147-153
<Go to ISI>://A1992HR96500006 AND http://www.botanischergarten.ch/EPOBIO/Beaudoin-Clonal-1992.pdf

Beck, L.C., Lessman, K.J., & Buker, R.J. (1975)
Inheritance of Pubescence and Its Use in Outcrossing Measurements between a Crambe-Hispanica Type and Crambe-Abyssinica Hochst Ex Fries,Rf. Crop Science, 15, 2, pp 221-224
<Go to ISI>://A1975W301100024 AND http://www.botanischergarten.ch/EPOBIO-Crambe/Beck-Pubescence-1975.pdf

Beckie, H.J., Hall, L.M., & Schuba, B. (2005)
Patch management of herbicide-resistant wild oat (Avena fatua). Weed Technology, 19, 3, pp 697-705
<Go to ISI>://000232674700027

Beckmann, J.S. & Soller, M. (1983)
Restriction Fragment Length Polymorphisms in Genetic-Improvement - Methodologies, Mapping and Costs. Theoretical and Applied Genetics, 67, 1, pp 35-43
<Go to ISI>://A1983RV54200005

Beer, S.C., Goffreda, J., Phillips, T.D., Murphy, J.P., & Sorrells, M.E. (1993)
Assessment of Genetic-Variation in Avena-Sterilis Using Morphological Traits, Isozymes, and Rflps. Crop Science, 33, 6, pp 1386-1393
<Go to ISI>://A1993MR71200051

Beilstein, M.A., Al-Shehbaz, I.A., & Kellogg, E.A. (2006)
Brassicaceae phylogeny and trichome evolution. American Journal of Botany, 93, 4, pp 607-619
<Go to ISI>://000236727400014

Bekkaoui, F., Mann, B., & Schroeder, B. (2003)
Application of DNA markers for the identification and management of hybrid poplar accessions. Agroforestry Systems, 59, 1, pp 53-59
<Go to ISI>://000185921100008

Bellagio Apomixis Declaration (1998)
Electronic Source: Declaration of the participants in the conference on "Designing a Research Strategy for Achieving Asexual Seed Production in Cereals," held at the Rockefeller Foundation's Bellagio Conference and Study Center (Italy), April 27th - May 1st, 1998, published by: CIMMYT
http://www.botanischergarten.ch/Apomixis/Bellagio-Apomixis-Delcaration.pdf AND
http://www.cimmyt.org/abc/ResearchProjects/Apomixis/apomixisnews10/htm/APOMIXISNews10-11.htm

Bergelson, J. & Purrington, C.B. (1996)
Surveying patterns in the cost of resistance in plants. American Naturalist, 148, 3, pp 536-558
<Go to ISI>://A1996VC52700005

Beri, S.M. & Anand, S.C. (1971)
Factors Affecting Pollen Shedding Capacity in Wheat. Euphytica, 20, 2, pp 327-&
<Go to ISI>://A1971J654900022 AMD http://www.botanischergarten.ch/EPOBIO-Wheat/Beri-Factors-1971.pdf

Besse, P. & McIntyre, C.L. (1999)

Chromosome in situ hybridisation of ribosomal DNA in Erianthus sect. Ripidium species with varying chromosome numbers confirms x = 10 in Erianthus sect. Ripidium. Genome, 42, 2, pp 270-273
<Go to ISI>://000080109800013

Besse, P., McIntyre, C.L., & Berding, N. (1997)
Characterisation of Erianthus sect Ripidium and Saccharum germplasm (Andropogoneae-Saccharinae) using RFLP markers. Euphytica, 93, 3, pp 283-292
<Go to ISI>://A1997WM17500004

Bitzer, M.J. & Patterso.Fl (1967)
Pollen Dispersal and Cross-Pollination of Soft Red Winter Wheat (Triticum Aestivum L). Crop Science, 7, 5, pp 482-&
<Go to ISI>://A1967A098200023

Blaringhem, L. (1921)
Research on the flax hybrids (Linum usitatissimum L.). Comptes Rendus Hebdomadaires Des Seances De L Academie Des Sciences, 173, pp 329-331
<Go to ISI>://000200947700121

Bonavent, J.F., Bessone, L., Geny, A., Berville, A., Denizot, J.P., & Brian, C. (1989)
A Possible Origin for the Sugar-Beet Cytoplasmic Male-Sterility Source Owen. Genome, 32, 2, pp 322-327
<Go to ISI>://A1989U744400024 AND http://www.botanischergarten.ch/EPOBIO-Beta/Bonavent-Origin-CMS-1989.pdf

Bond, J.M., Daniels, R., & Bioret, F. (2005)
Genetic diversity in Crambe maritima along the English Channel: the role of ocean currents in determining population structure. Ecography, 28, 3, pp 374-384
<Go to ISI>://000229428800010 AND http://www.botanischergarten.ch/EPOBIO-Crambe/Bond-Diversity-2005.pdf

Boudry, P., Morchen, M., Saumitoulaprade, P., Vernet, P., & Vandijk, H. (1993)
The Origin and Evolution of Weed Beets - Consequences for the Breeding and Release of Herbicide-Resistant Transgenic Sugar-Beets. Theoretical and Applied Genetics, 87, 4, pp 471-478
<Go to ISI>://A1993MM63000009 AND http://www.botanischergarten.ch/EPOBIO-Beta/Boudry-Origin-1993.pdf

Boudry, P., Wieber, R., Saumitoulaprade, P., Pillen, K., Vandijk, H., & Jung, C. (1994)
Identification of Rflp Markers Closely Linked to the Bolting Gene-B and Their Significance for the Study of the Annual Habit in Beets (Beta-Vulgaris L). Theoretical and Applied Genetics, 88, 6-7, pp 852-858
<Go to ISI>://A1994PB68100034

Boutin, V., Jean, R., Valero, M., & Vernet, P. (1988)
Gynodioecy in Beta-Maritima. Acta Oecologica-Oecologia Plantarum, 9, 1, pp 61-66
<Go to ISI>://A1988M874100006 AND NEBIS

Boutin, V., Pannenbecker, G., Ecke, W., Schewe, G., Saumitoulaprade, P., Jean, R., Vernet, P., & Michaelis, G. (1987)
Cytoplasmic Male-Sterility and Nuclear Restorer Genes in a Natural-Population of Beta-Maritima - Genetic and Molecular Aspects. Theoretical and Applied Genetics, 73, 5, pp 625-629
<Go to ISI>://A1987G802500001 AND http://www.botanischergarten.ch/EPOBIO-Beta/Boutin-CMS-Restorer-1987.pdf

BretagneSagnard, B., Fouilloux, G., & Chupeau, Y. (1996)
Induced albina mutations as a tool for genetic analysis and cell biology in flax (Linum usitatissimum). Journal of Experimental Botany, 47, 295, pp 189-194
<Go to ISI>://A1996UA12100005

Briard, M., Horvais, A., & Peron, J.Y. (2002)
Wild seakale (Crambe maritima L.) diversity as investigated by morphological and RAPD markers. Scientia Horticulturae, 95, 1-2, pp 1-12
<Go to ISI>://000178233200001 AND http://www.botanischergarten.ch/EPOBIO-Crambe/Briard-Diversity-2002.pdf

Brouwer, W., Stählin, I., & Caesar, K. (1976)
Beta. *In Handbuch des speziellen Pflanzenbaues,* (ed W. Brouwer), Vol. 2, pp. 188-368. Verlag Paul Parey, Berlin, Hamburg,

Brunner, A.M., Busov, V.B., & Strauss, S.H. (2004)
Poplar genome sequence: functional genomics in an ecologically dominant plant species. Trends in Plant Science, 9, 1, pp 49-56
<Go to ISI>://000188757600011

Brunner, A.M. & Nilsson, O. (2004)
Revisiting tree maturation and floral initiation in the poplar functional genomics era. New Phytologist, 164, 1, pp 43-51
<Go to ISI>://000223662000006

Brunsfeld, S.J.D., Soltis, D.E., & Soltis, P.S. (1992)
Evolutionary patterns and processes in Salix sect. Longifoliae: evidence from chloroplast DNA. Systematic Botany, 17, pp 239-256

Bruun, L., Haldrup, A., Petersen, S.G., Frese, L., deBock, T.S.M., & Lange, W. (1995)
Self-incompatibility reactions in wild species of the genus Beta and their relation to taxonomical classification and geographical origin. Genetic Resources and Crop Evolution, 42, 4, pp 293-301
<Go to ISI>://A1995TM72100002 AND NEBIS, but Vol 1995 missing, No.42 in 1994

Burdon, J.J., Thrall, P.H., & Brown, A.H.D. (1999)
Resistance and virulence structure in two Linum marginale-Melampsora lini host-pathogen metapopulations with different mating systems. Evolution, 53, 3, pp 704-716
<Go to ISI>://000081507300005

Busov, V.B., Meilan, R., Pearce, D.W., Ma, C.P., Rood, S.B., & Strauss, S.H. (2003)
Activation tagging of a dominant gibberellin catabolism gene (GA 2-oxidase) from poplar that regulates tree stature. Plant Physiology, 132, 3, pp 1283-1291
<Go to ISI>://000185076800020

Buttler, K. (1977)
Revision von Beta Sektion Corollinae (Chenopodiacea). I. Selbststerile Basisarten. Mitt. Bot. München, 13, pp 255-336

Cahn, E. (1925)
A study of fertility in some common varieties of wheat with respect to anther length and amount of pollen in parents and offspring. J. Am. Soc. Agron., 17, pp 591–595

Caldecott, R.J., Stevens, H., & Roberts, B.J. (1959)
Stein rust resistant variants in irradiated populations-mutations for field hybrids? Agronomy Journal, 51, pp 401-408

Caligari, P.D.S., Yapabandara, Y., Paul, E.M., Perret, J., Roger, P., & Dunwell, J.M. (1993)
Field Performance of Derived Generations of Transgenic Tobacco. Theoretical and Applied Genetics, 86, 7, pp 875-879
<Go to ISI>://A1993LX23000011

Campbell, S.C. & Mast, A.A. (1971)
Seed production. *In Advances in Sugar beet production: principles and pra* (eds R.T. Johnson, J.T. Alexander, G.E. Rush & G.W. Hawkes), pp. 437-450. Iowa State Univ. Press, Ames

Candolle, A.P.d. (1821) 'Regni Vegetabilis Systema Naturale 2 Treuttel andWurtz, Paris, pp

Candolle, A.P.d. (1824) Prodromus Systematis Naturalis Regni Vegetabilis 1 Treuttel andWurtz,, Strasbourg and London., pp

Cartea, M.E., Soengas, P., Picoaga, A., & Ordas, A. (2005)
Relationships among Brassica napus (L.) germplasm from Spain and Great Britain as determined by RAPD markers. Genetic Resources and Crop Evolution, 52, 6, pp 655-662
<Go to ISI>://000233351000002

Cassens, D.L. & Eslyn, W.E. (1983)
Field Trials of Chemicals to Control Sapstain and Mold on Yellow-Poplar and Southern Yellow Pine Lumber. Forest Products Journal, 33, 10, pp 52-56
<Go to ISI>://A1983RM40200007

Cauderon, Y. (1994)
Cytogénétique et amélioration des plantes : l´exemple des hybrides entre Triticum et Elytriga. C. R. Soc. Biol., 188, pp 93-107

Cavan, G., Cussans, J., & Moss, S. (2001)
Managing the risks of herbicide resistance in wild oat. Weed Science, 49, 2, pp 236-240
<Go to ISI>://000175241500015 AND http://www.botanischergarten.ch/EPOBIO-Avena/Cavan-Herbicide-Wildoat-2001.pdf

Cervera, M.T., Storme, V., Soto, A., Ivens, B., Van Montagu, M., Rajora, O.P., & Boerjan, W. (2005)
Intraspecific and interspecific genetic and phylogenetic relationships in the genus Populus based on AFLP markers. Theoretical and Applied Genetics, 111, 7, pp 1440-1456
<Go to ISI>://000233323900023 AND http://www.botanischergarten.ch/EPOBIO/Cervera-AFLP-2005.pdf

Chamberlain, D. & Stewart, C.N. (1999)
Transgene escape and transplastomics. Nature Biotechnology, 17, 4, pp 330-331
<Go to ISI>://000079574400015

Chaudhuri, B.K. & Sen, S. (1976)
Cytogenetics of Interspecific Hybrids in Genus Linum. Nucleus, 19, 1, pp 31-35
<Go to ISI>://A1976CA55600009

Chen, Y. & Dribnenki, P. (2002)
Effect of genotype and medium composition on flax Linum usitatissimum L. anther culture. Plant Cell Reports, 21, 3, pp 204-207
<Go to ISI>://000179204200003

Chen, Y.H., Chen, C., & Lo, C.C. (1993)
Studies on Anatomy and Morphology in Saccharum-Miscanthus Nobilized Hybrids .1. Transmission of Tillering, Ratooning, Adaptation and Disease Resistance from Miscanthus Spp. Journal of the Agricultural Association of China, 164, pp 31-45
<Go to ISI>://A1993MW79600003 NOT IN NEBIS

Cheung, W.Y., Champagne, G., Hubert, N., & Landry, B.S. (1997)
Comparison of the genetic maps of Brassica napus and Brassica oleracea. Theoretical and Applied Genetics, 94, 5, pp 569-582
<Go to ISI>://A1997WX38800004 AND http://www.botanischergarten.ch/Brassica/Cheung-ComparisonGeneitc-Maps-1997.pdf

Chevre, A.M., Adamczyk, K., Eber, F., Huteau, V., Coriton, O., Letanneur, J.C., Laredo, C., Jenczewski, E., & Monod, H. (2007)
Modelling gene flow between oilseed rape and wild radish. I. Evolution of chromosome structure. Theoretical and Applied Genetics, 114, 2, pp 209-221
<Go to ISI>://000242855700002 AND http://www.botanischergarten.ch/EPOBIO-Brassica/Chevre-Modelling-2007.pdf

Chevre, A.M., Eber, F., Barret, P., Dupuy, P., & Brace, J. (1997)
Identification of the different Brassica nigra chromosomes from both sets of B-oleracea B-nigra and B-napus B-nigra addition lines with a special emphasis on chromosome transmission and self-incompatibility. Theoretical and Applied Genetics, 94, 5, pp 603-611
<Go to ISI>://A1997WX38800008

Choi, C., Liu, Z.L., & Adams, K.L. (2006)
Evolutionary transfers of mitochondrial genes to the nucleus in the Populus lineage and coexpression of nuclear and mitochondrial Sdh4 genes. New Phytologist, 172, 3, pp 429-439
<Go to ISI>://000241238800007

Chou, C.H. & Lee, Y.F. (1991)
Allelopathic Dominance of Miscanthus-Transmorrisonensis in an Alpine Grassland Community in Taiwan. Journal of Chemical Ecology, 17, 11, pp 2267-2281
<Go to ISI>://A1991GR43400018 AND NEBIS

Chou, C.H. & Ueng, J.J. (1992)
Phylogenetic Relationship among Species of Miscanthus Populations in Taiwan. Botanical Bulletin of Academia Sinica, 33, 1, pp 63-73
<Go to ISI>://A1992HB45600008 ZENTRALBIBLIOTHEK ZÜRICH

Christopher, M.E., Miranda, M., Major, I.T., & Constabel, C.P. (2004)
Gene expression profiling of systemically wound-induced defenses in hybrid poplar. Planta, 219, 6, pp 936-947
<Go to ISI>://000224614600003

Clayton, W. & Renvoize, S. (1986)
Genera Graminum : grasses of the world. kew bulletin additional series, 13, pp 389

Clemente, M. & Hernandez-Bermejo, E. (1978a)
El aparato nectarıgeno de la tribu Brassiceae (Cruciferae). Anales Inst. Bot. Cavanilles, 35, pp 279-296

Clemente, M. & Hernandez-Bermejo, E. (1978b)
La corola de la tribu Brassiceae. Anales Inst. Bot. Cavanilles, 35, pp 297–334

Clemente, M. & Hernandez-Bermejo, E. (1980a)
Clasificacion jerarquica de las Brasiceas segun caracteres de las piezas esteriles de su flor. Anales Jard. Bot. Madrid, 36, pp 97–113

Clemente, M. & Hernandez-Bermejo, E. (1980b)
El caliz de la tribu Brassiceae (Cruciferae). Anales Jard. Bot. Madrid, 36, pp 77–96

Clifton-Brown, J.C., Lewandowski, I., Andersson, B., Basch, G., Christian, D.G., Kjeldsen, J.B., Jorgensen, U., Mortensen, J.V., Riche, A.B., Schwarz, K.U., Tayebi, K., & Teixeira, F. (2001)
Performance of 15 Miscanthus genotypes at five sites in Europe. Agronomy Journal, 93, 5, pp 1013-1019
<Go to ISI>://000170948000009 AND http://www.botanischergarten.ch/EPOBIO-Miscanthus/Clifton-Brown-Genotype-2001.pdf

Clifton-Brown, J.C., Stampfl, P.F., & Jones, M.B. (2004)
Miscanthus biomass production for energy in Europe and its potential contribution to decreasing fossil fuel carbon emissions. Global Change Biology, 10, 4, pp 509-518
<Go to ISI>://000220548800010 AND http://www.botanischergarten.ch/EPOBIO-Miscanthus/Clifton-Brown-Energy-2004.pdf

Cole, C.T. (2005)

Allelic and population variation of microsatellite loci in aspen (Populus tremuloides). New Phytologist, 167, 1, pp 155-164
<Go to ISI>://000229581600016

Coons, G. (1975)
Interspecific hybrids between B. vulgaris L. and the wild species of Beta. J Am Soc Sugar Beet Technol, 18, pp 281–306

Cornelius, J.A. & Simmons, E.A. (1969)
Crambe-Abyssinica - a New Commercial Oilseed. Tropical Science, 11, 1, pp 17-&
<Go to ISI>://A1969F353700001 AND http://www.botanischergarten.ch/EPOBIO-Crambe/Cornelius-Commercial-1969.pdf

Cronquist, A. (1988) The evolution and classification of flowering plants New York Botanical Garden, Bronx, New York, pp

Cross, R.H., McKay, S.A.B., McHughen, A.G., & Bonham-Smith, P.C. (2003)
Heat-stress effects on reproduction and seed set in Linum usitatissimum L. (flax). Plant Cell and Environment, 26, 7, pp 1013-1020
<Go to ISI>://000184065200004

Cseke, L.J., Cseke, S.B., Ravinder, N., Taylor, L.C., Shankar, A., Sen, B., Thakur, R., Karnosky, D.F., & Podila, G.K. (2005)
SEP-class genes in Populus tremuloides and their likely role in reproductive survival of poplar trees. Gene, 358, pp 1-16
<Go to ISI>://000232249500001

Czapik, R. (1999)
Enigma of apogamety. Protoplasma, 208, 1-4, pp 206-210
<Go to ISI>://000084535500024 AND http://www.botanischergarten.ch/Apomixis/Czapik-Enigma-Apogamety-1999.pdf

D'Souza, L. (1970)
Untersuchungen über die Eignung des Weizens als Pollenspender bei der Fremdbefruchtung, verglichen mit Roggen, Triticale und Secalotricum. Zeitschift für Pflanzenzüchtung, 63, pp 246-269

Damgaard, C. (2002)
Quantifying the invasion probability of genetically modified plants. BioSafety Journal, 7, 1, pp Paper 1
http://www.bioline.org.br/by

Damgaard, C. & Kjellsson, G. (2005)
Gene flow of oilseed rape (Brassica napus) according to isolation distance and buffer zone. Agriculture Ecosystems & Environment, 108, 4, pp 291-301
<Go to ISI>://000229661600001 AND http://www.botanischergarten.ch/EPOBIO-Brassica/Damgaard-Geneflow-2005.pdf

Daniell, H. (2002)
Molecular strategies for gene containment in transgenic crops. Nature Biotechnology, 20, 6, pp 581-586
<Go to ISI>://000175973500023 AND http://www.botanischergarten.ch/Apomixis/Daniell-Strategies-2002.pdf

Daniels, J. & Roach, B. (1987)
Taxonomy and evolution in breeding. *In Sugarcane improvement through breeding* (ed D. Heinz), pp. 7-84. Elsevier, Amsterdam

De Vries, A.P.D. (1971)
Flowering Biology of Wheat, Particularly in View of Hybrid Seed Production - Review. Euphytica, 20, 2, pp 152-&
<Go to ISI>://A1971J654900002 AND http://www.botanischergarten.ch/EPOBIO-Wheat/DeVries-Flowering-1971.pdf

De Vries, A.P.D. (1974a)
Some Aspects of Cross-Pollination in Wheat (Triticum-Aestivum L) .3. Anther Length and Number of Pollen Grains Per Anther. Euphytica, 23, 1, pp 11-19
<Go to ISI>://A1974S350200002

De Vries, A.P.D. (1974b)
Some Aspects of Cross-Pollination in Wheat (Triticum-Aestivum L) .4. Set on Male Sterile Plants as Influenced by Distance from Pollen Source, Pollinator - Male Sterile Ratio and Width of Male Sterile Strip. Euphytica, 23, 3, pp 601-622
<Go to ISI>://A1974U812000018 AND NEBIS

De Wet, J. (1981)
The evolution of weed beets in sugar beet populations. Die Kulturpflanze, 29, pp 301-310
not in WOS, not available in NEBIS

Demeke, T., Adams, R.P., & Chibbar, R. (1992)
Potential Taxonomic Use of Random Amplified Polymorphic DNA (Rapd) - a Case-Study in Brassica. Theoretical and Applied Genetics, 84, 7-8, pp 990-994
<Go to ISI>://A1992JT30000033

Desplanque, B., Boudry, P., Broomberg, K., Saumitou-Laprade, P., Cuguen, J., & Van Dijk, H. (1999)
Genetic diversity and gene flow between wild, cultivated and weedy forms of Beta vulgaris L-(Chenopodiaceae), assessed by RFLP and microsatellite markers. Theoretical and Applied Genetics, 98, 8, pp 1194-1201

<Go to ISI>://000081124700002 AND http://www.botanischergarten.ch/EPOBIO-Beta/Desplanque-Diversity-1999.pdf

Desplanque, B., Hautekeete, N., & Van Dijk, H. (2002)
Transgenic weed beets: possible, probable, avoidable? Journal of Applied Ecology, 39, 4, pp 561-571
<Go to ISI>://000177255000002 AND http://www.botanischergarten.ch/EPOBIO-Beta/Desplanque-possible-2002.pdf

Devaux, C., Lavigne, C., Austerlitz, F., & Klein, E.K. (2007)
Modelling and estimating pollen movement in oilseed rape (Brassica napus) at the landscape scale using genetic markers. Molecular Ecology, 16, 3, pp 487-499
<Go to ISI>://000243755800003 AND http://www.botanischergarten.ch/Brassica/Devaux-Modelling-Brassica-2007.pdf

Di-Giovanni, F., Beckett, P.M., & Flenley, J.R. (1989)
Modelling dispersion and deposition of tree pollen within a forest canopy. Grana, 28, pp 129–139

Di-Giovanni, F. & Kevan, P.G. (1991)
Factors affecting pollen dynamics and its importance to pollen contamination: A review. J. For. Res., 21, pp 1155–1170

Dias, J.S. (1995)
Genetic relationships of Portuguese coles and other close related Brassica genotypes using nuclear RFLPs. Genetic Resources and Crop Evolution, 42, 4, pp 363-369
<Go to ISI>://A1995TM72100009

Diederichsen, A. (2001)
Comparison of genetic diversity of flax (Linum usitatissimum L.) between Canadian cultivars and a world collection. Plant Breeding, 120, 4, pp 360-362
<Go to ISI>://000171356600017 AND http://www.botanischergarten.ch/EPOBIO-Linum/Diederichsen-Comparison-2001.pdf

Diers, B.W. & Osborn, T.C. (1994)
Genetic Diversity of Oilseed Brassica-Napus Germ Plasm Based on Restriction-Fragment-Length-Polymorphisms. Theoretical and Applied Genetics, 88, 6-7, pp 662-668
<Go to ISI>://A1994PB68100006

DiFazio, S. (2002)
Measuring and Modeling Gene Flow From Hybrid Poplar Plantations: Implications for Transgenic Risk Assessment. Doctor of Philosophy Thesis, Oregon State University, Corvallis, OR 97331-4501 541-737-1000 Thesis, pp 241
http://www.fsl.orst.edu/tgerc/dif_thesis/difaz_thesis.pdf

DiFazio, S.P., Slavov, G.T., Burczyk, J., Leonardi, S., & Strauss, S.H. (2004)
Gene flow from tree plantations and implications for transgenic risk assessment. *In Plantation Forest Biotechnology for the 21st Century* (eds C. Walter & M. Carson), pp. 405-422. Research Signpost, Kerala, India

Djerbi, S., Lindskog, M., Arvestad, L., Sterky, F., & Teeri, T.T. (2005)
The genome sequence of black cottonwood (Populus trichocarpa) reveals 18 conserved cellulose synthase (CesA) genes. Planta, 221, 5, pp 739-746
<Go to ISI>://000230490200013

Doering, D.S. (2004)
Will the marketplace see the sustainable forest for the transgenic trees? *In The Bioengineered Forest: Challenges to Science and Society,* (eds S.H. Strauss & H.D. Bradshaw), pp. 112–140. Resources for the Future, Washington DC

Donahue, R.A. & Michler, C.H. (1993)
Effect of Glyphosate on Chlorophyll Fluorescence in Transgenic Hybrid Poplar. Plant Physiology, 102, 1, pp 140-140
<Go to ISI>://A1993LD89000803

Dowding, P. (1987)
Wind pollination mechanisms and aerobiology. Int. Rev. Cytol., 107, pp 421–437

Doyle, J.J., Doyle, J.L., & Brown, A.H.D. (1990)
Chloroplast DNA Polymorphism and Phylogeny in the B-Genome of Glycine Subgenus Glycine (Leguminosae). American Journal of Botany, 77, 6, pp 772-782
<Go to ISI>://A1990DJ52600008

Driessen, S., Pohl, M., & Bartsch, D. (2001)
RAPD-PCR analysis of the genetic origin of sea beet (Beta vulgaris ssp maritima) at Germany's Baltic Sea coast. Basic and Applied Ecology, 2, 4, pp 341-349
<Go to ISI>://000172713900006 AND http://www.botanischergarten.ch/EPOBIO-Beta/Driessen-RAPD-PCR-2001.pdf

Dubey, D.K. & Singh, S.P. (1966)
Use of Cytoplasmic Male Sterility for Production of Hybrid Seeds in Flax (Linum Usitatissimum L). Crop Science, 6, 2, pp 125-&
<Go to ISI>://A19667816000006

Duvall, M.R. & Morton, B.R. (1996)
Molecular phylogenetics of poaceae: An expanded analysis of rbcL sequence data. Molecular Phylogenetics and Evolution, 5, 2, pp 352-358
<Go to ISI>://A1996UF78400007

Eastham, C. & Sweet, J. (2002)
Genetically modified organisms (GMOs): The significance of gene flow through pollen transfer, European Environment Agency pp 75 Copenhagen (Report)
http://reports.eea.eu.int/environmental_issue_report_2002_28/en

Eckenwalder, J.E. (1984a)
Natural Intersectional Hybridization between North-American Species of Populus (Salicaceae) in Sections Aigeiros and Tacamahaca .1. Population Studies of P X Parryi. Canadian Journal of Botany-Revue Canadienne De Botanique, 62, 2, pp 317-324
<Go to ISI>://A1984SH33800019

Eckenwalder, J.E. (1984b)
Natural Intersectional Hybridization between North-American Species of Populus (Salicaceae) in Sections Aigeiros and Tacamahaca .2. Taxonomy. Canadian Journal of Botany-Revue Canadienne De Botanique, 62, 2, pp 325-335
<Go to ISI>://A1984SH33800020

Eckenwalder, J.E. (1984c)
Natural Intersectional Hybridization between North-American Species of Populus (Salicaceae) in Sections Aigeiros and Tacamahaca .3. Paleobotany and Evolution. Canadian Journal of Botany-Revue Canadienne De Botanique, 62, 2, pp 336-342
<Go to ISI>://A1984SH33800021

Eckenwalder, J.E. (1996)
Taxonomic signal and noise in multivariate interpopulational relationships in Populus mexicana (Salicaceae). Systematic Botany, 21, 3, pp 261-271
<Go to ISI>://A1996WQ81700001 AND http://www.botanischergarten.ch/EPOBIO/Eckenwalder-Signals-Mexic-1996.pdf

Einspahr, D.W. & Winton, L.L. (1976)
Genetics of quaking aspen, Res. Pap. WO-25,. USDA For. Serv. Res. Pap. WO-25, 25, pp 23

Ellstrand, N. & Devlin, B. (1989)
Transmission Genetics of Isozyme Loci in Raphanus Sativus (Brassicaceae) - Stress-Dependent Non-Mendelian Segregation. American Journal of Botany, 76, 1, pp 40-46
<Go to ISI>://A1989R942500006

Ellstrand, N.C., Devlin, B., & Marshall, D.L. (1989)
Gene Flow by Pollen into Small Populations - Data from Experimental and Natural Stands of Wild Radish. Proceedings of the National Academy of Sciences of the United States of America, 86, 22, pp 9044-9047
<Go to ISI>://A1989AZ87100098

Evans, A. & Weir, J. (1981)
The evolution of weed beet in sugar beet crops. Kulturpflanze, 29, pp 301-310
in NEBIS nicht erhältlich

Falinski, J.B. (1998)
Androgyny of individuals and polygamy in populations of Salix myrsinifolia Salisb. in the south-western part of its geographical range (NE-Poland). Evolution and Systematics, 1, 2, pp 238-266
http://www.ingentaconnect.com/content/urban/291/1998/00000001/00000002/art00006 AND
http://dx.doi.org/10.1078/1433-8319-00061 AND http://www.botanischergarten.ch/EPOBIO-Salix/Falinski-Androgyny-1998.pdf

Fang, X.H., Gu, S.H., Xu, Z.Y., Chen, F., Guo, D.D., Zhang, H.B., & Wu, N.H. (2004)
Construction of a binary BAC library for an apomictic monosomic addition line of Beta corolliflora in sugar beet and identification of the clones derived from the alien chromosome. Theoretical and Applied Genetics, 108, 7, pp 1420-1425
<Go to ISI>://000220988400028 AND http://www.botanischergarten.ch/EPOBIO-Beta/Fang-Apomixis-2004.pdf

Fargue, A., Colbach, N., Pierre, J., Picault, H., Renard, M., & Meynard, J.M. (2006)
Predictive study of the advantages of cleistogamy in oilseed rape in limiting unwanted gene flow. Euphytica, 151, 1, pp 1-13
<Go to ISI>://000241578700001 AND http://www.botanischergarten.ch/EPOBIO-Brassica/Fargue-Predictive-2006.pdf

Farmer, R.E., Jr. & Pitcher, J.A. (1981)
Pollen handling for Southern hardwoods. *In Pollen management handbook* (ed E. Franklin), Vol. Agriculture Handbook 587, pp. 77-83. USDA, Washington DC

Farrar, J. (1995) Trees in Canada. Fitzhenry & Whiteside/Canadian Forest Service, Markham and Ottawa, pp

Fechner, G.H., Burr, K.E., & Myers, J.F. (1981)
Effects of Storage, Temperature, and Moisture Stress on Seed-Germination and Early Seedling Development of Trembling Aspen. Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere, 11, 3, pp 718-722
<Go to ISI>://A1981MH96000038

Feldman, M., Lupton, F.G.H., & Miller, T.E. (1995)
Wheats. Triticum spp. (Graminae, Triticinae). *In Evolution of crop plants.* (eds J. Smart & N.W. Simmonds), Vol. 2nd edition, pp. 184-192. Longman Scientific and Technical,

Fenart, S., Touzet, P., Arnaud, J.F., & Cuguen, J. (2006)
Emergence of gynodioecy in wild beet (Beta vulgaris ssp maritima L.): a genealogical approach using chloroplastic nucleotide sequences. Proceedings of the Royal Society B-Biological Sciences, 273, 1592, pp 1391-1398
<Go to ISI>://000237780100012 AND http://www.botanischergarten.ch/EPOBIO-Beta/Fénart-Gynodioecy-2006.pdf

Ferguson, A.W., Fitt, B.D.L., & Williams, I.H. (1997)
Insect injury to linseed in south-east England. Crop Protection, 16, 7, pp 643-652
<Go to ISI>://A1997YE81900005

Ferrant, V. & Bouharmont, J. (1994)
Origin of Gynogenetic Embryos of Beta-Vulgaris L. Sexual Plant Reproduction, 7, 1, pp 12-16
<Go to ISI>://A1994MW79000003

Firbank, L.G., Heard, M.S., Woiwod, I.P., Hawes, C., Haughton, A.J., Champion, G.T., Scott, R.J., Hill, M.O., Dewar, A.M., Squire, G.R., May, M.J., Brooks, D.R., Bohan, D.A., Daniels, R.E., Osborne, J.L., Roy, D.B., Black, H.I.J., Rothery, P., & Perry, J.N. (2003)
An introduction to the Farm-Scale Evaluations of genetically modified herbicide-tolerant crops. Journal of Applied Ecology, 40, 1, pp 2-16
http://www.botanischergarten.ch/Farmscale/Firbanks-Introduction-2003.pdf

Fladung, M. & Kumar, S. (2002)
Gene stability in transgenic aspen-populus - III. T-DNA repeats influence transgene expression differentially among different transgenic lines. Plant Biology, 4, 3, pp 329-338
<Go to ISI>://000176426800005 AND http://www.botanischergarten.ch/EPOBIO/Fladung-Stability-2002.pdf

Fladung, M., Nowitzki, O., Ziegenhagen, B., & Kumar, S. (2003)
Vegetative and generative dispersal capacity of field released transgenic aspen trees. Trees-Structure and Function, 17, 5, pp 412-416
<Go to ISI>://000184902500005 AND http://www.botanischergarten.ch/EPOBIO/Fladung-Dispersal-2003.pdf

Flannery, M.-L., Meade, C., & Mullins, E. (2005)
Employing a composite gene-flow index to numerically quantify a crop's potential for gene flow: an Irish perspective. Environ. Biosafety Res., 4, pp 29-43
http://www.edpsciences.org/ebr AND http://www.botanischergarten.ch/Africa-Harvest-Geneflow/Flannery-ebr0418.pdf

Fontana, F., Lazzeri, L., Malaguti, L., & Galletti, S. (1998)
Agronomic characterization of some Crambe abyssinica genotypes in a locality of the Po Valley. European Journal of Agronomy, 9, 2-3, pp 117-126
<Go to ISI>://000077569900006 AND http://www.botanischergarten.ch/EPOBIO-Crambe/Fontana-Agronomic-1998.pdf

Ford, C.S., Allainguillaume, J., Grilli-Chantler, P., Cuccato, G., Allender, C.J., & Wilkinson, M.J. (2006)
Spontaneous gene flow from rapeseed (Brassica napus) to wild Brassica oleracea. Proceedings of the Royal Society B-Biological Sciences, 273, 1605, pp 3111-3115
<Go to ISI>://000242684800012

Foster, R., Pooni, H.S., & Mackay, I.J. (1998)
Quantitative analysis of Linum usitatissimum crosses for dual-purpose traits. Journal of Agricultural Science, 131, pp 285-292
<Go to ISI>://000077142700005

Francisco-Ortega, J., Fuertes-Aguilar, J., Gomez-Campo, C., Santos-Guerra, A., & Jansen, R.K. (1999)
Internal transcribed spacer sequence phylogeny of Crambe L. (Brassicaceae): Molecular data reveal two Old World disjunctions. Molecular Phylogenetics and Evolution, 11, 3, pp 361-380
<Go to ISI>://000079862100003 AND http://www.botanischergarten.ch/EPOBIO-Crambe/Francisco-Ortega-SpacerSequence-1999.pdf

Franco, J. (1964)
Populus L. *In Flora Europaea* (eds T. Tutin, V. Heywood, H. Burges, D. Valentine, S. Walters & D. Webb), Vol. 1, pp. 54-55. Cambridge University Press, Cambridge http://rbg-web2.rbge.org.uk/FE/fe.html

Free, J.B., Williams, I.H., Longden, P.C., & Johnson, M.G. (1975)
Insect Pollination of Sugar-Beet (Beta-Vulgaris) Seed Crops. Annals of Applied Biology, 81, 2, pp 127-134
<Go to ISI>://A1975AT00700001 AND NEBIS

Frese, L. (1991)
Variation Patterns in a Leaf Beet (Beta-Vulgaris, Chenopodiaceae) Germplasm Collection. Plant Systematics and Evolution, 176, 1-2, pp 1-10
<Go to ISI>://A1991FN24600001 AND http://www.botanischergarten.ch/EPOBIO-Beta/Frese-Germplasm-Patterns-1991.pdf

Frese, L. (2003)
Sugar beets and related wild species – from collecting to utilisation. Schriften zu Genetischen Ressourcen, ?, pp 170-181
http://www.botanischergarten.ch/EPOBIO-Beta/Frese-Taxonomy-2003.pdf

Frese, L., Desprez, B., & Ziegler, D. (2001a)
Potential of genetic resources and breeding strategies for base-broadening in Beta. *In Broadening the Genetic Base of Crop Production* (eds H.D. Cooper, C. Spillane & T. Hodgkin), pp. 295–309. IPGRI/FAO, Rome
http://www.botanischergarten.ch/EPOBIO-Beta/Frese-Potential-IPGRI-2002.pdf

Frese, L., Ziegler, D., & Rau, R.K. (2001b)
A taxonomic guide for wild and cultivated beets (Beta L.). pp
http://www.fal.de/bgrc/eu9542 (not functional) AND http://www.botanischergarten.ch/EPOBIO-Beta/Frese-Taxonomy-Beet-web-2001.pdf

Friedt, W., Bickert, C., & Schaub, H. (1995)
In-Vitro Breeding of High-Linolenic, Doubled-Haploid Lines of Linseed (Linum-Usitatissimum L) Via Androgenesis. Plant Breeding, 114, 4, pp 322-326
<Go to ISI>://A1995TB22200009

Frietema, D.V.F. (1994)
Botanical files on lettuce (Lactuca sativa L.) for gene flow between wild and cultivated lettuce (Lactuca sativa L. Including L. Serriola L., Compositae) and the generalized implications for risk assessment on Genetically Modified Plants. Gorteria, supplement 2, pp 1-44

Frietema, D.V.F. (1996)
Cultivated plants and the wild flora. Effect analysis by dispersal codes, University of Leiden, Leiden Thesis, pp 100

Fu, Y.B., Diederichsen, A., Richards, K.W., & Peterson, G. (2002)
Genetic diversity within a range of cultivars and landraces of flax (Linum usitatissimum L.) as revealed by RAPDs. Genetic Resources and Crop Evolution, 49, 2, pp 167-174
<Go to ISI>://000174482300007 AND http://www.botanischergarten.ch/EPOBIO-Linum/Fu-Diversity-2002.pdf

Gaget, M., Villar, M., Kerhoas, C., & Dumas, C. (1989)
Sexual Reproduction in Populus .2. Information Molecules of the Pollen Grain. Annales Des Sciences Forestieres, 46, pp S67-S71
<Go to ISI>://A1989CU99900015 AND http://www.botanischergarten.ch/EPOBIO/Gaget-Reproduction-1989.pdf

Gao, D. & Jung, C. (2002)
Monosomic addition lines of Beta corolliflora in sugar beet: plant morphology and leaf spot resistance. Plant Breeding, 121, 1, pp 81-86
<Go to ISI>://000174286300014

Garcke, A. (1972) Illustrierte Flora Paul Parey Verlag, Berlin, pp

Gebhard, F. & Smalla, K. (1999)
Monitoring field releases of genetically modified sugar beets for persistence of transgenic plant DNA and horizontal gene transfer. Fems Microbiology Ecology, 28, 3, pp 261-272
<Go to ISI>://000079122100007 AND http://www.botanischergarten.ch/HorizontalGT/Gebhard-Monitoring-HGT-1999.pdf

Geisler, G. (1991) Farbatlas Landwirtschaftlicher Kulturpflanzen Eugen Ulmer Verlag, Stuttgart, Germany, pp

Genissel, A., Leple, J.C., Millet, N., Augustin, S., Jouanin, L., & Pilate, G. (2003)
High tolerance against Chrysomela tremulae of transgenic poplar plants expressing a synthetic cry3Aa gene from Bacillus thuringiensis ssp tenebrionis. Molecular Breeding, 11, 2, pp 103-110
<Go to ISI>://000181041000003

Ghaemi, M., Sarrafi, A., & Alibert, G. (1993)
Influence of Genotype and Culture Conditions on the Production of Embryos from Anthers of Tetraploid Wheat (Triticum-Turgidum). Euphytica, 65, 2, pp 81-85
<Go to ISI>://A1993KW94200001

Gill, K.S. & Yermanos, D.M. (1967a)
Cytogenetic Studies on Genus Linum .2. Hybrids among Taxa with 9 as Haploid Chromosome Number. Crop Science, 7, 6, pp 627-&
<Go to ISI>://A1967A406100022

Gill, K.S. & Yermanos, D.M. (1967b)
Cytogenetic Studies on Genus Linum .I. Hybrids among Taxa with 15 as Haploid Chromosome Number. Crop Science, 7, 6, pp 623-&
<Go to ISI>://A1967A406100021

Glaszmann, J.C., Dufour, P., Grivet, L., Dhont, A., Deu, M., Paulet, F., & Hamon, P. (1997)
Comparative genome analysis between several tropical grasses. Euphytica, 96, 1, pp 13-21
<Go to ISI>://A1997XU40800003 AND http://www.botanischergarten.ch/Africa-Harvest-Sorghum-Lit-1/Glaszmann-Genome-1997.pdf

Gomez-Campo, C. (1980)
Morphology and morpho-taxonomy of the tribe Brassiceae. *In Brassica Crops and Wild Allies* (eds S. Tsunoda, K. Hinata & C. Gomez-Campo), pp. 3-30. Japan Sci. Press,, Tokyo

Gomez-Campo, C. & Tortosa, M.E. (1974)
The taxonomic and evolutionary significance of some juvenile characters in the Brassiceae. Bot. J. Linn. Soc., 69, pp 105–124

Gonzalez-Andujar, J.L. & Fernandezquintanilla, C. (1993)
Strategies for the Control of Avena-Sterilis in Winter-Wheat Production Systems in Central Spain. Crop Protection, 12, 8, pp 617-623
<Go to ISI>://A1993MH91400009

Gonzalez-Andujar, J.L. & Perry, J.N. (1995)
Models for the Herbicidal Control of the Seed Bank of Avena-Sterilis - the Effects of Spatial and Temporal Heterogeneity and of Dispersal. Journal of Applied Ecology, 32, 3, pp 578-587
<Go to ISI>://A1995RQ01500012 AND http://www.botanischergarten.ch/EPOBIO-Avena/Gonzales-Andjurar-Seedbank-1995.pdf

Goodspeed, T.H. (1954) The Genus Nicotiana Waltham, Massachusetts, pp

Gorshkova, T.A., Carpita, N.C., Chemikosova, S.B., Kuz'mina, G.G., Kozhevnikov, A.A., & Lozovaya, V.V. (1998)
Galactans are a dynamic component of flax cell walls. Russian Journal of Plant Physiology, 45, 2, pp 234-239
<Go to ISI>://000072598000014

Gorshkova, T.A., Sal'nikova, V.V., Chemikosova, S.B., Ageeva, M.V., Pavlencheva, N.V., & van Dam, J.E.G. (2003)
The snap point: a transition point in Linum usitatissimum bast fiber development. Industrial Crops and Products, 18, 3, pp 213-221
<Go to ISI>://000186360100002

Gorshkova, T.A., Salnikov, V.V., Pogodina, N.M., Chemikosova, S.B., Yablokova, E.V., Ulanov, A.V., Ageeva, M.V., Van Dam, J.E.G., & Lozovaya, V.V. (2000)
Composition and distribution of cell wall phenolic compounds in flax (Linum usitatissimum L.) stem tissues. Annals of Botany, 85, 4, pp 477-486
<Go to ISI>://000086478100007

Graef, F., Zughart, W., Hommel, B., Heinrich, U., Stachow, U., & Werner, A. (2005)
Methodological scheme for designing the monitoring of genetically modified crops at the regional scale. Environmental Monitoring and Assessment, 111, 1-3, pp 1-26
<Go to ISI>://000233532400001 AND http://www.botanischergarten.ch/EPOBIO-Beta/Graef-Monitoring-Beta-2005.pdf

Grant, M.C., Mitton, J.B., & Linhart, Y.B. (1992)
Even Larger Organisms. Nature, 360, 6401, pp 216-216
<Go to ISI>://A1992JY96000034

Grassl, C.O. (1972)
Taxonomy of Saccharum relatives: Sclerostachya, Narenga, and Erianthus, Proceedings of 14th Congress, ISSCT,, Ed. pp 240–248

Grebenstein, B., Roser, M., Sauer, W., & Hemleben, V. (1998)
Molecular phylogenetic relationships in Aveneae (Poaceae) species and other grasses as inferred from ITS1 and ITS2 rDNA sequences. Plant Systematics and Evolution, 213, 3-4, pp 233-250
<Go to ISI>://000078583100008 AND http://www.botanischergarten.ch/EPOBIO-Avena/Grebenstein-Systematics-1998.pdf

Greef, J.M. & Deuter, M. (1993)
Syntaxonomy of Miscanthus-X-Giganteus Greef-Et-Deu. Angewandte Botanik, 67, 3-4, pp 87-90
<Go to ISI>://A1993MA81500001 AND http://www.botanischergarten.ch/EPOBIO-Miscanthus/Greef-Syntaxonomy-1993.pdf

Green, A. (1983)

Interspecific hybridisation in the genus Linum., Adelaide, South Australia, Australian plant breeding conference 14.-18.February 1983, Ed. C. Driscoll pp

Gressel, J. & Al-Ahmad, H. (2005)
Assessing and managing biological risks of plants used for bioremediation, including risks of transgene flow. Zeitschrift für Naturforschung C-a Journal of Biosciences, 60, 3-4, pp 154-165
<Go to ISI>://000230152300002 AND http://www.botanischergarten.ch/Modelling/Gressel-Mitigation-2005.pdf

Greuter, W., McNeill, J., Barrie, F., Burdet, H., Demoulin, V., Figueiras, S., Nicolson, D., Silva, P., Skog, J., Trehane, P., Turland, J., & Hawksworth, D. (2000)
International Code of Botanical Nomenclature (St. Louis Code). In Regnum Vegetabile, Vol. 138. Koeltz Scientific Books, Königstein

Grindeland, R.I. & Frohberg, R.C. (1966)
Outcrossing of Oat Plants (Avena Sativa L) Grown from Mutagen-Treated Seeds. Crop Science, 6, 4, pp 381-&
<Go to ISI>://A19668185600031 AND http://www.botanischergarten.ch/EPOBIO-Avena/Grindeland-Outcrossing-1966.pdf

Grossniklaus, U., Nogler, G.A., & van Dijk, P.J. (2001)
How to avoid sex: The genetic control of gametophytic apomixis. Plant Cell, 13, 7, pp 1491-1497
<Go to ISI>://000170061400002 AND http://www.botanischergarten.ch/Apomixis/Grossniklaus-Apomixis-2001.pdf

Guadagnuolo, R., Bianchi, D., & Felber, F. (2001a)
Specific genetic markers for wheat, spelt, and four wild relatives: comparison of isozymes, RAPDs, and wheat microsatellites. Genome, 44, 4, pp 610-621
<Go to ISI>://000170177800014

Guadagnuolo, R., Savova-Bianchi, D., & Felber, F. (2001b)
Gene flow from wheat (Triticum aestivum L.) to jointed goatgrass (Aegilops cylindrica Host.), as revealed by RAPD and microsatellite markers. Theoretical and Applied Genetics, 103, 1, pp 1-8
<Go to ISI>://000170129000001

Gustafson, D.I., Horak, M.J., Rempel, C.B., Metz, S.G., Gigax, D.R., & Hucl, P. (2005)
An empirical model for pollen-mediated gene flow in wheat. Crop Science, 45, 4, pp 1286-1294
<Go to ISI>://000230713700013 http://www.botanischergarten.ch/EPOBIO-Wheat/Gustafson-Wheat-Model-2005.pdf

Gustafson, D.L., Horak, M.J., Metz, S.G., Gigax, D.R., Rempel, C.B., & Hucl, R. (2006)
Comments on "An empirical model for pollen-mediated gene flow in wheat" (Crop Sci. 45 : 1286-1294). Crop Science, 46, 2, pp 1019-1019
<Go to ISI>://000235991100091 AND http://www.botanischergarten.ch/EPOBIO-Wheat/Willienborg-Response-Gustafson.pdf

Hallden, C., Karlsson, G., Lind, C., Moller, I.M., & Heneen, W.K. (1991)
Microsporogenesis and Tapetal Development in Fertile and Cytoplasmic Male-Sterile Sugar-Beet (Beta-Vulgaris L). Sexual Plant Reproduction, 4, 3, pp 215-225
<Go to ISI>://A1991FY20800011

Hamilton, C.M., Frary, A., Lewis, C., & Tanksley, S.D. (1996)
Stable transfer of intact high molecular weight DNA into plant chromosomes. Proceedings of the National Academy of Sciences of the United States of America, 93, 18, pp 9975-9979
<Go to ISI>://A1996VF61400118

Hamilton, C.M., Frary, A., Xu, Y.M., Tanksley, S.D., & Zhang, H.B. (1999)
Construction of tomato genomic DNA libraries in a binary-BAC (BIBAC) vector. Plant Journal, 18, 2, pp 223-229
<Go to ISI>://000080474500011

Hardig, T.M., Brunsfeld, S.J., Fritz, R.S., Morgan, M., & Orians, C.M. (2000)
Morphological and molecular evidence for hybridization and introgression in a willow (Salix) hybrid zone. Molecular Ecology, 9, 1, pp 9-24
<Go to ISI>://000085368000002 AND http://www.botanischergarten.ch/EPOBIO-Salix/Hardig-Introgression-2000.pdf

Harding, K. & Harris, P.S. (1994)
Risk assessment of the release of genetically modified plants : a review, Edited by Ministry of Agriculture, Fisheries and Food, pp 54 London (Report)

Harlan, J. & Zohary, D. (1966)
Distribution of wild wheats and barley. Science, 153, pp 1074-1080

Harlan, J.R. (1992) Crops and Man, 2nd edition American Society of Agronomy, Madison, Wisconsin, IS: 0-89118-107-5, pp 295

Harrington, J.B. (1932)
Natural crossing in wheat, oats and barley at Official Journal of the European Union. 2003. Regulation (EC) No.Saskatoon, Saskatchewan. Sci. Agric., 12, pp 470–483

Hayek von, A. (1911)
Entwurf eines Cruciferensystems auf phylogenetischer Grundlage. Beihefte Botanisches Centralblatt, 27, pp 127-335

Hellum, A.K. (1973)
Seed Storage and Germination of Black Poplar. Canadian Journal of Plant Science, 53, 1, pp 227-228
<Go to ISI>://A1973O780300040

Helm, J. (1957)
Versuch einer morphologisch-systematischen Gliederung von Beta vulgaris L. Züchter, 27, pp 203-222
http://www.botanischergarten.ch/EPOBIO-Beta/Helm-Morpologisch-1953.pdf

Herschbach, C. & Kopriva, S. (2002)
Transgenic trees as tools in tree and plant physiology. Trees-Structure and Function, 16, 4-5, pp 250-261
<Go to ISI>://000176081700002 AND http://www.botanischergarten.ch/EPOBIO/Herschbach-transgenic-Trees-2002.pdf

Heslop-Harrison, J. (1979)
An interpretation of the hydrodynamics of pollen. J. Am. Soc. Bot., 66, pp 737–743

Hetterscheid, W.L.A., Van Ettekoven, C., Van den Berg, R.G., & Brandenburg, W.A. (1999)
Cultonomy in statutory registration exemplified by Allium L-crops. Plant Varieties and Seeds, 12, 3, pp 149-160
<Go to ISI>://000085747600002 NOT IN NEBIS

Hetterscheid, W.L.A., VandenBerg, R.G., & Brandenburg, W.A. (1996)
An annotated history of the principles of cultivated plant classification. Acta Botanica Neerlandica, 45, 2, pp 123-134
<Go to ISI>://A1996UT37300002 AND http://www.botanischergarten.ch/EPOBIO-Beta/Hetterscheid-Cultonomy-1996.pdf

Hickey, L. & Wolfe, J.A. (1975)
The bases of angiosperm phylogeny: vegetative morphology. Ann. Missouri Bot. Gard., 2, pp 538-589

Hillmann, G. (1996)
'Late Pleistocene changes in wild food plants available to huntergatherers of the northern Fertile Crescent:possible preludes to cereal cultivation. *In The Origin and Spread of Agriculture and Pastoralism in Eurasia,* (ed D.R. Harris), pp. 159–203, p 189. University College Press, London

Himmelsbach, D.S., Khalili, S., & Akin, D.E. (1998)
FT-IR microspectroscopic imaging of flax (Linum usitatissimum L.) stems. Cellular and Molecular Biology, 44, 1, pp 99-108
<Go to ISI>://000072781500012

Hodkinson, T.R., Chase, M.W., Lledo, M.D., Salamin, N., & Renvoize, S.A. (2002a)
Phylogenetics of Miscanthus, Saccharum and related genera (Saccharinae, Andropogoneae, Poaceae) based on DNA sequences from ITS nuclear ribosomal DNA and plastid trnL intron and trnL-F intergenic spacers. Journal of Plant Research, 115, 1121, pp 381-392
<Go to ISI>://000178919500009 AND http://www.botanischergarten.ch/EPOBIO-Miscanthus/Hodkinson-Phylogenetics-2002.pdf

Hodkinson, T.R., Chase, M.W., & Renvoize, S.A. (2002b)
Characterization of a genetic resource collection for Miscanthus (Saccharinae, Andropogoneae, Poaceae) using AFLP and ISSR PCR. Annals of Botany, 89, 5, pp 627-636
<Go to ISI>://000175542000016 AND http://www.botanischergarten.ch/EPOBIO-Miscanthus/Hodkinson-Characterization-2002.pdf

Hodkinson, T.R., Chase, M.W., Takahashi, C., Leitch, I.J., Bennett, M.D., & Renvoize, S.A. (2002c)
The use of DNA sequencing (ITS and trnL-F), AFLP, and fluorescent in situ hybridization to study allopolyploid Miscanthus (Poaceae). American Journal of Botany, 89, 2, pp 279-286
<Go to ISI>://000178098100012 AND http://www.botanischergarten.ch/EPOBIO-Miscanthus/Hodkinson-DNA-Alloploid-2002.pdf

Hodkinson, T.R. & Renvoize, S.A. (2001)
Nomenclature of Miscanthus x giganteus (Poaceae). Kew bulletin, 56, pp 757-758

Hoenicka, H. & Fladung, M. (2006)
Biosafety in Populus spp. and other forest trees: from non-native species to taxa derived from traditional breeding and genetic engineering. Trees-Structure and Function, 20, 2, pp 131-144
<Go to ISI>://000235251100001 AND http://www.botanischergarten.ch/EPOBIO/Hoenicka-Biosafety-2006.pdf

Hojland, J.G. & Pedersen, S. (1994)
Sugarbeet, Beetroot and Fodder Beet (Beta vulgaris L. subsp. vulgaris) : Dispersal, establishement and interactions with the environment, The National Forest and Nature Agency pp 73 Copenhagen (Report)

Hornsey, K.G. (1973a)
Attempted Pollen-Transmission of Cytoplasmic Male Sterility and Spontaneous Occurrence of Male Sterility in O-Type Lines of Sugar-Beet (Beta-Vulgaris L). Theoretical and Applied Genetics, 43, 1, pp 31-34

<Go to ISI>://A1973P183900007 AND

Hornsey, K.G. (1973b)
Occurrence of Hexaploid Plants among Autotetraploid Populations of Sugar-Beet, (Beta-Vulgaris L) and Production of Tetraploid Progeny Using a Diploid Pollinator. Caryologia, 26, 2, pp 225-228
<Go to ISI>://A1973R810800006 AND http://www.botanischergarten.ch/EPOBIO-Beta/Hornsey-Transmission-1973.pdf

Hornsey, K.G. (1975)
Exploitation of Polyploidy in Sugar-Beet Breeding. Journal of Agricultural Science, 84, JUN, pp 543-557
<Go to ISI>://A1975AF06200021

Hornsey, K.G. & Arnold, M.H. (1979)
Origins of Weed Beet. Annals of Applied Biology, 92, 2, pp 279-285
<Go to ISI>://A1979HD68200015 AND http://www.botanischergarten.ch/EPOBIO-Beta/Hornsey-Origins-1979.pdf

Hoshikawa, K. (1960)
Studies on the reopen floret in wheat. (in Japanese). Proc. Crop. Sci. Soc., 29, pp 103–106

Hsiao, C., Chatterton, N.J., Asay, K.H., & Jensen, K.B. (1994)
Phylogenetic-Relationships of 10 Grass Species - an Assessment of Phylogenetic Utility of the Internal Transcribed Spacer Region in Nuclear Ribosomal DNA in Monocots. Genome, 37, 1, pp 112-120
<Go to ISI>://A1994MY29000014

Hsiao, C., Chatterton, N.J., Asay, K.H., & Jensen, K.B. (1995a)
Molecular Phylogeny of the Pooideae (Poaceae) Based on Nuclear Rdna (Its) Sequences. Theoretical and Applied Genetics, 90, 3-4, pp 389-398
<Go to ISI>://A1995QR66400013

Hsiao, C., Chatterton, N.J., Asay, K.H., & Jensen, K.B. (1995b)
Phylogenetic-Relationships of the Monogenomic Species of the Wheat Tribe, Triticeae (Poaceae), Inferred from Nuclear Rdna (Internal Transcribed Spacer) Sequences. Genome, 38, 2, pp 211-223
<Go to ISI>://A1995QX26100003

Hsiao, C., Jacobs, S.W.L., Barker, N.P., & Chatterton, N.J. (1998)
A molecular phylogeny of the subfamily Arundinoideae (Poaceae) based on sequences of rDNA. Australian Systematic Botany, 11, 1, pp 41-52
<Go to ISI>://000072971700005

Hsiao, C., Jacobs, S.W.L., Chatterton, N.J., & Asay, K.H. (1999)
A molecular phylogeny of the grass family (Poaceae) based on the sequences of nuclear ribosomal DNA (ITS). Australian Systematic Botany, 11, 5-6, pp 667-688
<Go to ISI>://000080058500002

Hucl, P. (1996)
Out-crossing rates for 10 Canadian spring wheat cultivars. Can. J. Plant Sci., 76, pp 423-427

Hucl, P. & Matus-Cadiz, M. (2001)
Isolation distances for minimizing out-crossing in spring wheat. Crop Science, 41, 4, pp 1348-1351
<Go to ISI>://000170881200052

Imam, A.G. & Allard, R.W. (1965)
Population Studies in Predominantly Self-Pollinated Species .6. Genetic Variability between and within Natural Populations of Wild Oats from Differing Habitats in California. Genetics, 51, 1, pp 49-&
<Go to ISI>://A19656051100005 AND http://www.botanischergarten.ch/EPOBIO-Avena/Imam-Wildoat-Populations-1964.pdf

Imbert, E. & Lefevre, F. (2003)
Dispersal and gene flow of Populus nigra (Salicaceae) along a dynamic river system. Journal of Ecology, 91, 3, pp 447-456
<Go to ISI>://000183144700011 AND http://www.botanischergarten.ch/EPOBIO/Imbert-Dispersal-2003.pdf

Inaba, R. & Nishio, T. (2002)
Phylogenetic analysis of Brassiceae based on the nucleotide sequences of the S-locus related gene, SLR1. Theoretical and Applied Genetics, 105, 8, pp 1159-1165
<Go to ISI>://000179982700008 AND http://www.botanischergarten.ch/EPOBIO-Crambe/Inaba-Phylogenetic-2002.pdf

International Plant Names Index (2004)
Electronic Source: International Plant Names Index, (ed R.B.G.a. Kew), http://www.ipni.org AND http://www.botanischergarten.ch/EPOBIO-Crambe/Crambe-IPN-2007.pdf

Ivanov, M.K., Revenko, A.S., & Dymshits, G.M. (2004)

Cytoplasmic male sterility-associated structural variation of the mitochondrial genome regions containing rps3 and orf215 in sugar beet Beta vulgaris L. Molecular Biology, 38, 3, pp 345-350
<Go to ISI>://000222291900005 AND http://www.botanischergarten.ch/EPOBIO-Beta/Ivanov-CMS-2004.pdf

Ivanov, M.K., Revenko, A.S., Maletskaya, E.I., Maletskii, S.I., & Dymshits, G.M. (2005)
Structural and transcriptional variation of mitochondrial DNA in pollen-sterile agamospermous sugar beet (Beta vulgaris L.) progeny. Russian Journal of Genetics, 41, 11, pp 1245-1253
<Go to ISI>://000234424300008 AND http://www.botanischergarten.ch/EPOBIO-Beta/Ivanov-mitochondrial-DNA-2005.pdf

Jackson, J.F. & Clarke, G.R. (1991)
Gene Flow in an Almond Orchard. Theoretical and Applied Genetics, 82, 2, pp 169-173
<Go to ISI>://A1991GB09800007

Jacot, Y., Ammann, K., Rufener Al Mazyad, P., Chueca, C., Davin, J., Gressel, J., Loureiro, I., Wang, H., & Benavente, E. (2004)
Hybridization between wheat and wild relatives, a European Union research programme. *In Introgression from Genetically Modified Plants into Wild Relatives* (eds H. den Nijs, D. Bartsch & J. Sweet), pp. 63-74. CABI Publishing
http://www.botanischergarten.ch/Geneflow/Jacot-et-al-Amsterdam-2003.pdf

Janchen, E. (1947)
Das System der Cruciferen. Oesterr. Bot. Zeitschr., 91, pp 1-28

Jarosz, A.M. & Burdon, J.J. (1991)
Host-Pathogen Interactions in Natural-Populations of Linum-Marginale and Melampsora-Lini .2. Local and Regional Variation in Patterns of Resistance and Racial Structure. Evolution, 45, 7, pp 1618-1627
<Go to ISI>://A1991GR18400006

Jassem, B. (1976)
Embryology and Genetics of Apomixis in Section Corollinae of Genus Beta. Acta Biologica Cracoviensia Series Botanica, 19, 2, pp 149-&
<Go to ISI>://A1976CZ96200006

Jassem, B. (1992)
Species relationships in the genus Beta as revealed by crossing experiments, Braunschweig, Germany International Crop Network Series No. 7. IBPGR, Rome. pp. 55-61., International Beta Genetic Resources Network.A report on the 2nd International Beta Genetic Resources Workshop held at the Institute for Crop Science and Plant Breeding June 1991, Ed. L. Frese pp 24-28 and 55-61

Jassem, M. & Jassem, B. (1971)
Apomixis in Some Beta Species. Genetica Polonica, 12, 3, pp 217-&
<Go to ISI>://A1971L738900015

Jeanmonod, D. & Schlussel, A. (2003)
Notes and contributions on Corsican flora, XIX. Candollea, 58, 2, pp 273-287
<Go to ISI>://000187963300001

Jensen, N.F. (1966)
Genetics and inheritance in oats. Agronomy, 8, pp 125-206

Jensen, N.F. (1968)
Results of a survey on isolation requirements for wheat. Annu. Wheat Newsl., 15, pp 26-28

Jing, Z.P., Gallardo, F., Pascual, M.B., Sampalo, R., Romero, J., de Navarra, A.T., & Canovas, F.M. (2004)
Improved growth in a field trial of transgenic hybrid poplar overexpressing glutamine synthetase. New Phytologist, 164, 1, pp 137-145
<Go to ISI>://000223662000014

Johannessen, M.M., Damgaard, C., Andersen, B.A., & Jorgensen, R.B. (2006)
Competition affects the production of first backcross offspring on F-1-hybrids, Brassica napus x B-Rapa. Euphytica, 150, 1-2, pp 17-25
<Go to ISI>://000240396100002 AND http://www.botanischergarten.ch/EPOBIO-Brassica/Johannessen-Competition-2006.pdf

Johansson, H., Sterky, F., Amini, B., Lundeberg, J., & Kleczkowski, L.A. (2002)
Molecular cloning and characterization of a cDNA encoding poplar UDP-glucose dehydrogenase, a key gene of hemicellulose/pectin formation. Biochimica Et Biophysica Acta-Gene Structure and Expression, 1576, 1-2, pp 53-58
<Go to ISI>://000176060700007

Jones, J., Shlumukov, L., Carland, F., English, J., Scofield, S., Bishop, G., & Harrison, K. (1992)
Effective vectors for transformation, expression of heterologous genes, and assaying transposon excision in transgenic plants. Transgenic Research, V1, 6, pp 285-297
http://dx.doi.org/10.1007/BF02525170

Jones, M.B. & Walsh, M., eds. (2001)
Miscanthus – for Energy and Fibre, James and James (Science Publishers), London,

Jonsell, B. (1982)
Cruciferae. *In Flora of Tropical East Africa* (ed R.M. Polhill). Balkema, Rotterdam

Joppa, L.R., McNeal, F.H., & Berg, M.A. (1968)
Pollen Production and Pollen Shedding of Hard Red Spring (Triticum Aestivum L Em Thell) and Durum (T Durum Desf) Wheats. Crop Science, 8, 4, pp 487-&
<Go to ISI>://A1968B664400028 AND http://www.botanischergarten.ch/EPOBIO-Wheat/Joppa-Production-1968.pdf

Jorgensen, R.B. & Andersen, B. (1994)
Spontaneous Hybridization between Oilseed Rape (Brassica-Napus) and Weedy Brassica-Campestris (Brassicaceae) - a Rise of Growing Genetically-Modified Oilseed Rape. American Journal of Botany, 81, 12, pp 1620-1626
<Go to ISI>://A1994PZ95600014

Jouanin, L., Bonade-Bottino, M., Girard, C., Morrot, G., & Giband, M. (1998)
Transgenic plants for insect resistance. Plant Science, 131, 1, pp 1-11
<Go to ISI>://000072021300001

Jung, C., Pillen, K., Frese, L., Fahr, S., & Melchinger, A.E. (1993)
Phylogenetic-Relationships between Cultivated and Wild-Species of the Genus Beta Revealed by DNA Fingerprinting. Theoretical and Applied Genetics, 86, 4, pp 449-457
<Go to ISI>://A1993LE22300008 AND NEBIS

Kearns, C.A. & Inouye, D.W. (1994)
Fly Pollination of Linum-Lewisii (Linaceae). American Journal of Botany, 81, 9, pp 1091-1095
<Go to ISI>://A1994PG63000002

Kehr, W.R. (1973)
Cross-Fertilization of Alfalfa as Affected by Genetic Markers, Planting Methods, Locations, and Pollinator Species. Crop Science, 13, 3, pp 296-298
<Go to ISI>://A1973Q094300002

Kellogg, E.A. (1992)
Tools for Studying the Chloroplast Genome in the Triticeae (Gramineae) - an Ecori Map, a Diagnostic Deletion, and Support for Bromus as an Outgroup. American Journal of Botany, 79, 2, pp 186-197
<Go to ISI>://A1992HE97400012

Kemperman, J.A. & Barnes, B.V. (1976)
Clone Size in American Aspens. Canadian Journal of Botany-Revue Canadienne De Botanique, 54, 22, pp 2603-2607
<Go to ISI>://A1976CM85200012 AND http://www.botanischergarten.ch/EPOBIO/Kemperman-Clone-1976.pdf

Khalilov, I.I. (1991a)
Generis Crambe L. (Cruciferae) sectiones tres novae. Novosti Sist. Vyssh. Rast., 28, pp 78-79

Khalilov, I.I. (1991b)
The system of the genus Crambe (Brassicaceae). Bot. Zhurn.(St. Petersburg), 76, pp 1612–1613

Khan, M.N., Heyne, E.G., & Arp, A.L. (1973)
Pollen Distribution and Seedset on Triticum-Aestivum L. Crop Science, 13, 2, pp 223-226
<Go to ISI>://A1973P509100020

Khan, M.N., Heyne, E.G., & Goss, J.A. (1971)
Effect of Relative Humidity on Viability and Longevity of Wheat Pollen. Crop Science, 11, 1, pp 125-&
<Go to ISI>://A1971I561000046

Kherde, M.K., Atkins, I.M., Merkle, O.G., & Porter, K.B. (1967)
Cross Pollination Studies with Male Sterile Wheats of 3 Cytoplasms Seed Size on F1 Plants and Seed and Anther Size of 45 Pollinators. Crop Science, 7, 4, pp 389-&
<Go to ISI>://A19679817300034

Khvorostov, I.B., Ivanov, M.K., Morozov, I.V., & Dymshits, G.M. (2001)
A cytoplasmic male sterility-associated rearrangement of the mitochondrial cob gene region in sugar beet Beta vulgaris L. Molecular Biology, 35, 5, pp 699-701
<Go to ISI>://000171958700010 NEBIS not available

Kihara, H. (1962)
Comparative Gene Analysis in Wheat and Its Relatives. Japanese Journal of Genetics, 37, 5, pp 363-&
<Go to ISI>://A19627454B00064

Kihara, H. (1965)

Origin of Wheat in Light of Comparative Genetics. Japanese Journal of Genetics, 40, 1, pp 45-&
<Go to ISI>://A19656425700006 http://www.botanischergarten.ch/Wheat/Kihara-Origin-Wheat-1965.pdf

Kimura, M. (1928)
U¨ ber Toisusu, eine neue Salicaceen-Gattung und die systematische Stellung derselben. Botanical Magazine (Tokyo), 48, pp 287–290

Kimura, M. (1980)
A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution, 16, pp 111–120

Kimura, M. (1988)
De salicis subgenere Pleuradenia commentatio. Science Reports of the Tohoku University, Fourth Series, Biology, 39, pp 143-147

Klopfenstein, N.B., Allen, K.K., Avila, F.J., Heuchelin, S.A., Martinez, J., Carman, R.C., Hall, R.B., Hart, E.R., & McNabb, H.S. (1997)
Proteinase inhibitor II gene in transgenic poplar: Chemical and biological assays. Biomass & Bioenergy, 12, 4, pp 299-311
<Go to ISI>://A1997XL87800009

Klopfenstein, N.B., Shi, N.Q., Kernan, A., McNabb, H.S., Hall, R.B., Hart, E.R., & Thornburg, R.W. (1991)
Transgenic Populus Hybrid Express a Wound-Inducible Potato Proteinase Inhibitor-Ii - Cat Gene Fusion. Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere, 21, 9, pp 1321-1328
<Go to ISI>://A1991GE32200004

Kmec, P. & Weiss, M.J. (1997)
Seasonal abundance of diamondback moth (Lepidoptera: Yponomeutidae) on Crambe abyssinica. Environmental Entomology, 26, 3, pp 483-488
<Go to ISI>://A1997XJ22900002 AND http://www.botanischergarten.ch/EPOBIO-Crambe/Kmec-Diamondback-1997.pdf

Koch, M., Al-Shehbaz, I.A., & Mummenhoff, K. (2003)
Molecular systematics, evolution, and population biology in the mustard family (Brassicaceae). Annals of the Missouri Botanical Garden, 90, 2, pp 151-171
<Go to ISI>://000183643300002 AND http://www.botanischergarten.ch/EPOBIO-Brassica/Koch-Systematics-2003.pdf

Koltunow, A.M. & Grossniklaus, U. (2003)
Apomixis: A developmental perspective. Annual Review of Plant Biology, 54, pp 547-574
<Go to ISI>://000185094100022 AND http://www.botanischergarten.ch/Apomixis/Koltunov-Apomixis-Developmental-2003.pdf

Konzak, C.F. (1959)
Radiation-induced mutations for stem rust resistance in oats. Agronomy Journal, 51, pp 518-520

Körber-Grohne, U. (1988) Nutzpflanzen in Deutschland - Kulturgeschichte und Biologie Theiss Verlag, Stuttgart, Germany, pp

Kostoff, D. (1943) Cytogenetics of the Genus Nicotiana. Karyosystematics, Genetics, Cytology, Cytogenetics and Phylesis of Tobaccos. State Printing House, Sofia, pp

Kowarik, I. (1992a)
Einf¨uhrung und Ausbreitung nichteinheimischer Geh¨olzarten in Berlin und Brandenburg. Verh Bot Ver Berlin Brandenburg Beih, 3, pp 188

Kowarik, I. (1992b)
Einführung und Ausbreitung nichteinheimischer Gehölzarten in Berlin und Brandenburg. Verh Bot Ver Berlin Brandenburg Beih, 3, pp 188

Kowarik, I. (1995)
Time-lags in biological invasions. *In Plant invasions:general aspects and special problems* (eds P. Pysek, K. Prach, M. Rejmanek & W. Wade), pp. 15'38. SPB Academic Publ, Amsterdam

Kowarik, I. (1999)
Ecological aspects of the release of transgenic trees- experiences from biological invasions,, Humboldt University, Berlin, , Proceedings “Release of transgenic trees- present achievements, problems, future prospects, Ed. pp 66'73

Kowarik, I. (2003a) Biologische Invasionen: Neophyten und Neozoen in Mitteleuropa. Eugen Ulmer, Stuttgart (Hohenheim), pp

Kowarik, I. (2003b)
Human agency in biological invasions: secondary releases foster naturalization and population expansion of alien plant species. Biological Invasions, 5, pp 293-312

Kowarik, I. (2005)
Urban ornamentals escaped from cultivation. *In Crop Ferality and Volunteerism* (ed J. Gressel). CRC Press, Boca Raton

Krüssmann, G. (1985) Manual of cultivated broad-leaved trees and shrubs., Translation from 1977 edn. II, E - PRO Timber Press, Portland, pp

Kübler, E. (1994) Weizenanbau Eugen Ulmer Verlag, Ulm, Germany, pp

Kubo, T., Nishizawa, S., & Mikami, T. (1999)
Alterations in organization and transcription of the mitochondrial genome of cytoplasmic male sterile sugar beet (Beta vulgaris L.). Molecular and General Genetics, 262, 2, pp 283-290
<Go to ISI>://000082852000010

Kumar, S. (2006)
Apomixis revisited. Current Science, 90, 3, pp 277-278
<Go to ISI>://000235497600004 AND http://www.botanischergarten.ch/EPOBIO-Miscanthus/Kumar-Apomixis-2006.pdf

Kumar, S. & Fladung, M. (2001)
Gene stability in transgenic aspen (Populus). II. Molecular characterization of variable expression of transgene in wild and hybrid aspen. Planta, 213, 5, pp 731-740
<Go to ISI>://000171254700008 AND http://www.botanischergarten.ch/EPOBIO/Kumar-Stability-2001.pdf

Lamboy, W.F. (1994a)
The Accuracy of the Maximum Parsimony Method for Phylogeny Reconstruction with Morphological Characters. Systematic Botany, 19, 4, pp 489-505
<Go to ISI>://A1994PT33900001

Lamboy, W.F. (1994b)
Computing Genetic Similarity Coefficients from Rapd Data - Correcting for the Effects of Pcr Artifacts Caused by Variation in Experimental Conditions. Pcr-Methods and Applications, 4, 1, pp 38-43
<Go to ISI>://A1994RM62500007

Lamboy, W.F. (1994c)
Computing Genetic Similarity Coefficients from Rapd Data - the Effects of Pcr Artifacts. Pcr-Methods and Applications, 4, 1, pp 31-37
<Go to ISI>://A1994RM62500006

Lange, W., Brandenburg, W.A., & De Bock, T.S.M. (1999)
Taxonomy and cultonomy of beet (Beta vulgaris L.). Botanical Journal of the Linnean Society, 130, 1, pp 81-96
<Go to ISI>://000080284500007 AND http://www.botanischergarten.ch/EPOBIO-Beta/Lange-Cultonomy-1999.pdf

Lanner, C. (1998)
Relationships of wild Brassica species with chromosome number 2n = 18, based on comparison of the DNA sequence of the chloroplast intergenic region between trnL (UAA) and trnF (GAA). Canadian Journal of Botany-Revue Canadienne De Botanique, 76, 2, pp 228-237
<Go to ISI>://000073722300007

Lanner, C., Bryngelsson, T., & Gustafsson, M. (1997)
Relationships of wild Brassica species with chromosome number 2n=18, based on RFLP studies. Genome, 40, 3, pp 302-308
<Go to ISI>://A1997XF12500004

Laporte, V., Viard, F., Bena, G., Valero, M., & Cuguen, J. (2001)
The spatial structure of sexual and cytonuclear polymorphism in the gynodioecious Beta vulgaris ssp maritima: I - at a local scale. Genetics, 157, 4, pp 1699-1710
<Go to ISI>://000168223400027

Latva-Karjanmaa, T., Suvanto, L., Leinonen, K., & Rita, H. (2006)
Sexual reproduction of european aspen (Populus tremula L.) at prescribed burned site: The effects of moisture conditions. New Forests, 31, 3, pp 545-558
<Go to ISI>://000237441700016 AND http://www.botanischergarten.ch/EPOBIO/Latva-Karianmaa-Reproduction-2006.pdf

Laurie, D.A., Griffiths, S., Dunford, R.P., Christodoulou, V., Taylor, S.A., Cockram, J., Beales, J., & Turner, A. (2004)
Comparative genetic approaches to the identification of flowering time genes in temperate cereals. Field Crops Research, 90, 1, pp 87-99
<Go to ISI>://000224357900008

Lazaro, A. & Aguinagalde, I. (1998)
Genetic diversity in Brassica oleracea L. (Cruciferae) and wild relatives (2n = 18) using isozymes. Annals of Botany, 82, 6, pp 821-828
<Go to ISI>://000077513100016

Leclerc, M.Y., Thurtell, G.W., & Kidd, G.E. (1988)

Measurements and Langevin Simulations of Mean Tracer Concentration Fields Downwind from a Circular Line Source inside an Alfalfa Canopy. Boundary-Layer Meteorology, 43, 3, pp 287-308
<Go to ISI>://A1988M862700006

Leflon, M., Eber, F., Letanneur, J.C., Chelysheva, L., Coriton, O., Huteau, V., Ryder, C.D., Barker, G., Jenczewski, E., & Chevre, A.M. (2006)
Pairing and recombination at meiosis of Brassica rapa (AA) x Brassica napus (AACC) hybrids. Theoretical and Applied Genetics, 113, 8, pp 1467-1480
<Go to ISI>://000241798000008 AND http://www.botanischergarten.ch/EPOBIO-Brassica/Leflon-Pairing-2006.pdf

Legere, A. (2005)
Risks and consequences of gene flow from herbicide-resistant crops: canola (Brassica napus L) as a case study. Pest Management Science, 61, 3, pp 292-300
<Go to ISI>://000227342100012 AND http://www.botanischergarten.ch/EPOBIO-Brassica/Legere-Brassica-Geneflow-2005.pdf

Legere, A., Simard, M.J., Johnson, E., Stevenson, F.C., Beckie, H., & Blackshaw, R.E. (2006)
Control of volunteer canola with herbicides: Effects of plant growth stage and cold acclimation. Weed Technology, 20, 2, pp 485-493
<Go to ISI>://000238476300032

Legg, B.J. & Powell, F.A. (1979)
Spore Dispersal in a Barley Crop - Mathematical-Model. Agricultural Meteorology, 20, 1, pp 47-67
<Go to ISI>://A1979GJ96900005

Leggett, J.M., Perret, S.J., Harper, J., & Morris, P. (2000)
Chromosomal localization of cotransformed transgenes in the hexaploid cultivated oat Avena sativa L. using fluorescence in situ hybridization. Heredity, 84, 1, pp 46-53
<Go to ISI>://000086165000006 AND http://www.botanischergarten.ch/EPOBIO-Avena/Legget-Avena-transgenic-2000.pdf

Legionnet, A., FaivreRampant, P., Villar, M., & Lefevre, F. (1997)
Sexual and asexual reproduction in natural stands of Populus nigra. Botanica Acta, 110, 3, pp 257-263
<Go to ISI>://A1997XL09600010 AND http://www.botanischergarten.ch/EPOBIO/Legionnet-Reproduction-1997.pdf

Lei, B., Li-Chan, E.C.Y., Oomah, B.D., & Mazza, G. (2003)
Distribution of cadmium-binding components in flax (Linum usitatissimum L.) seed. Journal of Agricultural and Food Chemistry, 51, 3, pp 814-821
<Go to ISI>://000180578300045

Leighty, C.E. & Sandok, W.J. (1924)
The blooming of wheat flowers.(German). J. Agricultural Research, 27, pp 231-317

Lelley, J. (1966)
Observation on the biology of fertilization with regard to seed production in hybrid wheat. Der Züchter (Genetic and Breeding research), 36, pp 314-317

Leple, J.C., Bonadebottino, M., Augustin, S., Pilate, G., Letan, V.D., Delplanque, A., Cornu, D., & Jouanin, L. (1995)
Toxicity to Chrysomela-Tremulae (Coleoptera, Chrysomelidae) of Transgenic Poplars Expressing a Cysteine Proteinase-Inhibitor. Molecular Breeding, 1, 4, pp 319-328
<Go to ISI>://A1995TK04600002

Lescot, M., Rombauts, S., Zhang, J., Aubourg, S., Mathe, C., Jansson, S., Rouze, P., & Boerjan, W. (2004)
Annotation of a 95-kb Populus deltoides genomic sequence reveals a disease resistance gene cluster and novel class I and class II transposable elements. Theoretical and Applied Genetics, 109, 1, pp 10-22
<Go to ISI>://000221936300002

Leseberg, C.H., Li, A.L., Kang, H., Duvall, M., & Mao, L. (2006)
Genome-wide analysis of the MADS-box gene family in Populus trichocarpa. Gene, 378, pp 84-94
<Go to ISI>://000240028000010

Lessman, K.J. & Meier, V.D. (1972)
Agronomic Evaluation of Crambe as a Source of Oil. Crop Science, 12, 2, pp 224-&
<Go to ISI>://A1972M322900024 AND http://www.botanischergarten.ch/EPOBIO-Crambe/Lessmann-Agronomic-1972.pdf

Lester, D.T. (1963)
Variation in sex expression in Populus tremuloides Michx. Silvae Genetica, 12, pp 141-151

Letschert, I. (1993)
Beta section Beta: biogeographical patterns of variation and taxonomy. WAU Dissertation, University of Wageningen, Wageningen Thesis, pp 1-153 (or 137?)
http://library.wur.nl/wda/abstracts/ab1595.html

Levin, D.A. & Kerster, H.W. (1974)
Gene Flow in Seed Plants. Evolutionary Biology, 7, pp 139-220

Lewandowski, I., Clifton-Brown, J.C., Scurlock, J.M.O., & Huisman, W. (2000)
Miscanthus: European experience with a novel energy crop. Biomass & Bioenergy, 19, 4, pp 209-227
<Go to ISI>://000165551300001 AND EZB

Lewandowskia, I., Clifton-Brownb, J., & Scurlock, J.M.O. (2000)
Miscanthus: European experience with a novel energy crop. 19, 4, pp 209-227
http://www.sciencedirect.com/science/article/B6V22-41M3H0T-1/2/0edf73794793a26a5c8069fcccf134be AND
http://www.botanischergarten.ch/EPOBIO-Miscanthus/Lewandowski-European-2000.pdf

Lexer, C., Fay, M.F., Joseph, J.A., Nica, M.S., & Heinze, B. (2005)
Barrier to gene flow between two ecologically divergent Populus species, P alba (white poplar) and P tremula (European aspen): the role of ecology and life history in gene introgression. Molecular Ecology, 14, 4, pp 1045-1057
<Go to ISI>://000227721900012 AND http://www.botanischergarten.ch/EPOBIO/Lexer-Barrier-2005.pdf

Li, H., Wengt, S., Shamgk, C., & Yang, P.C. (1961)
Cytological studies of sugarcane and its relatives. XVIII. Trigeneric hybrids of Saccharum officinarum L., Sc!erostachya fusca A. Camus, and Miscanthus japonicus Andersson. Botanical bulletin of Academia Sinica 2, pp 1-9

Linde-Laursen, I. (1993)
Cytogenetic Analysis of Miscanthus-Giganteus, an Interspecific Hybrid. Hereditas, 119, 3, pp 297-300
<Go to ISI>://A1993MV20300010 AND http://www.botanischergarten.ch/EPOBIO/Linde-Laursen-Giganteus-1993.pdf

Linnaeus, C. (1753) Species Plantarum 1, Holmiae, Stockholm, pp p. 222

Lisson, S.N. & Mendham, N.J. (2000)
Agronomic studies of flax (Linum usitatissimum L.) in south-eastern Australia. Australian Journal of Experimental Agriculture, 40, 8, pp 1101-1112
<Go to ISI>://000165971800006

Liu, Y.G., Shirano, Y., Fukaki, H., Yanai, Y., Tasaka, M., Tabata, S., & Shibata, D. (1999)
Complementation of plant mutants with large genomic DNA fragments by a transformation-competent artificial chromosome vector accelerates positional cloning. Proceedings of the National Academy of Sciences of the United States of America, 96, 11, pp 6535-6540
<Go to ISI>://000080527100112 AND http://www.botanischergarten.ch/EPOBIO-Beta/Liu-Complementation-1999.pdf

Liu, Z.Q., Adamczyk, K., Manzanares-Dauleux, M., Eber, F., Lucas, M.O., Delourme, R., Chevre, A.M., & Jenczewski, E. (2006)
Mapping PrBn and other quantitative trait loci responsible for the control of homeologous chromosome pairing in oilseed rape (Brassica napus L.) haploids. Genetics, 174, 3, pp 1583-1596
<Go to ISI>://000242532600044

Livers, R.W. (1964)
Seed yields of field-grown male-sterile wheats subjected to wind-borne pollen. Agronomy Abstracts, pp 72

Longden, P.C., Scott, R.K., & Wood, D.W. (1974)
Grading Monogerm Sugar-Beet Seed and Its Influence on Performance. Journal of Agricultural Science, 83, AUG, pp 125-133
<Go to ISI>://A1974T965400018 AND http://www.botanischergarten.ch/EPOBIO-Beta/Longden-insects-1975.pdf

Lorenz, M., Weihe, A., & Borner, T. (1994)
DNA Fragments of Organellar Origin in Random Amplified Polymorphic DNA (Rapd) Patterns of Sugar-Beet (Beta-Vulgaris L). Theoretical and Applied Genetics, 88, 6-7, pp 775-779
<Go to ISI>://A1994PB68100023

Lorenz, M., Weihe, A., & Borner, T. (1997)
Cloning and sequencing of RAPD fragments amplified from mitochondrial DNA of male-sterile and male-fertile cytoplasm of sugar beet (Beta vulgaris L). Theoretical and Applied Genetics, 94, 2, pp 273-278
<Go to ISI>://A1997WJ91200017 AND http://www.botanischergarten.ch/EPOBIO-Beta/Lorenz-Cloning-RAPD-1997.pdf

Loureiro, I., Escorial, M.C., Garcia-Baudin, J.M., & Chueca, M.C. (2001)
Capacidad de Potential de diffusion de genes de trigo a espexcies afines a traves del polen, Actas Congreso SMEH, Ed. pp 129-133

Lu, A.Z., Zhao, H., Wang, T.Y., & Wang, H.B. (2002)
Study of possibility of target gene introgresssion from transgenic wheat into non-transgenic plants through pollen. Acta Agriculturae Boreali-Sinica, 17, pp 16

Lu, C.S., Sharma, H.C., & Ohm, H.W. (1991)

Wheat Anther Culture - Effect of Genotype and Environmental-Conditions. Plant Cell Tissue and Organ Culture, 24, 3, pp 233-236
<Go to ISI>://A1991FF62900011

Lundqvist, A., Osterbye, U., Larsen, K., & Lindelau.Ib. (1973)
Complex Self-Incompatibility Systems in Ranunculus-Acris L and Beta-Vulgaris L. Hereditas, 74, 2, pp 161-168
<Go to ISI>://A1973R187800001 AND NEBIS

Lynch, J.M., Benedetti, A., Insam, H., Nuti, M.P., Smalla, K., Torsvik, V., & Nannipieri, P. (2004)
Microbial diversity in soil: ecological theories, the contribution of molecular techniques and the impact of transgenic plants and transgenic microorganisms. Biology and Fertility of Soils, 40, 6, pp 363-385
<Go to ISI>://000225303700001

Lysak, M.A. & Lexer, C. (2006)
Towards the era of comparative evolutionary genomics in Brassicaceae. Plant Systematics and Evolution, 259, 2-4, pp 175-198
<Go to ISI>://000238998800006 AND http://www.botanischergarten.ch/EPOBIO-Brassica/Lysak-Comparative-Genomics-2006.pdf

Lysgaard, C.P. (1991)
Froalderens indflydelse pä spireevne, spiringsenergi, platevakst og det endelige udbytte hos bederoe og kälroe samt pä anden generation af kälroe. Tidsskrift for plateavl, 95, pp 367-374

MacFadden, E.D. & Sears, E.R. (1946)
The origin of Triticum spelta and its free-threshing hexaploid relatives. Journal of Heredity, 37, 3 and 4, pp 81-89, and 106-116

Majewska-Sawka, A., Rodriguezgarcia, M.I., Nakashima, H., & Jassen, B. (1993)
Ultrastructural Expression of Cytoplasmic Male-Sterility in Sugar-Beet (Beta-Vulgaris L). Sexual Plant Reproduction, 6, 1, pp 22-32
<Go to ISI>://A1993KT06800004 AND http://www.botanischergarten.ch/EPOBIO-Beta/Majewska-Sawka-CMS-Ultrastructural-1993.pdf

Major, D.J. (1980)
Environmental effects on flowering. *In Hybridization of crop plants* (eds W.R. Fehr & H.H. Hadley), pp. 1-15. ASA and CSSA,, Madison, WI

Makarevitch, I., Svitashev, S.K., & Somers, D.A. (2003)
Complete sequence analysis of transgene loci from plants transformed via microprojectile bombardment. Plant Molecular Biology, 52, 2, pp 421-432
<Go to ISI>://000183204500015 AND http://www.botanischergarten.ch/EPOBIO-Avena/Makarevitch-Complete-Sequence-2003.pdf

Mandy, G. (1970) Pflanzenzüchtung - Kurz und bündig. VEB Deutscher Landwirtschafts-verlag,, Berlin, pp

Mansfeld, R. (1986) Verzeichnis landwirtschaftlicher und gärtnerischer Kulturpflanzen (ohne Zierpflanzen) vol. 1, 2nd ed. Springer Verlag,, Berlin, Heidelberg, New York., pp

Marhold, K. & Lihova, J. (2006)
Polyploidy, hybridization and reticulate evolution: lessons from the Brassicaceae. Plant Systematics and Evolution, 259, 2-4, pp 143-174
<Go to ISI>://000238998800005 AND http://www.botanischergarten.ch/EPOBIO-Brassica/Marhold-Polyploidy-2006.pdf

Marques, K., Sarazin, B., Chane-Favre, L., Zivy, M., & Thiellement, H. (2001)
Comparative proteomics to establish genetic relationships in the Brassicaceae family. Proteomics, 1, 11, pp 1457-1462
<Go to ISI>://000172680000013

Martin, F. & Kohler, A. (2004)
Structural and functional genomics of the poplar. Biofutur, 247, pp 38-42
<Go to ISI>://000224079100007

Martin, F., Tuskan, G.A., DiFazio, S.P., Lammers, P., Newcombe, G., & Podila, G.K. (2004)
Symbiotic sequencing for the Populus mesocosm. New Phytologist, 161, 2, pp 330-335
<Go to ISI>://000187550700004

Martin, M.T. & Garcia, J.A. (1991)
Plum Pox Potyvirus Rna Replication in a Crude Membrane-Fraction from Infected Nicotiana-Clevelandii Leaves. Journal of General Virology, 72, pp 785-790
<Go to ISI>://A1991FG44800005

McDonough, W.T. (1985)
Quaking aspen - seed germination and early seedling growth. Res. Pap. INT-234, USDA For. Serv., Ogden, UT, pp 13

McDougall, G.J., Goodman, B.A., & Chudek, J.A. (1992)
Nuclear-Magnetic-Resonance (Nmr) Microimaging of Stems of Linum-Usitatissimum. Journal of Agricultural Science, 119, pp 157-164
<Go to ISI>://A1992JR64600002

McHughen, A., M., J., & McSheffrey, S. (1990)
Two years of transgenic flax field tests; what to they tell us? *In Progress in Plant Cellular and Molecular Biology* (eds N.J.J. Nijkamp, v.d.P. L.H.W. & J. van Artrijk), pp. 207-212. Kluwer, Dordrecht

McHughen, A. & Rowland, G.G. (1991)
The Effect of T-DNA on the Agronomic Performance of Transgenic Flax Plants. Euphytica, 55, 3, pp 269-275
<Go to ISI>://A1991GM84300009

McKenzie, R.H.I., Brunner, H., Hsieh, S.C., & Mikaelsen, K. (1975)
Outcrossing rates in wheat, oats and barley produced from mutagen treated seeds. SABRAO, 7, pp 79-83

McLean, M.A. & Charest, P.J. (2000)
The regulation of transgenic trees in North America. Silvae Genetica, 49, 6, pp 233-239
<Go to ISI>://000169022300001

Meeuse, A.D.J. (1975)
Taxonomic Relationships of Salicaceae and Flacourtiaceae - Their Bearing on Interpretative Floral Morphology and Dilleniid Phylogeny. Acta Botanica Neerlandica, 24, 5-6, pp 437-457
<Go to ISI>://A1975BR37400008

Meilan, R., Auerbach, D.J., Ma, C., DiFazio, S.P., & Strauss, S.H. (2002a)
Stability of herbicide resistance and GUS expression in transgenic hybrid poplars (Populus sp.) during four years of field trials and vegetative propagation. Hortscience, 37, 2, pp 277-280
<Go to ISI>://000175099700004

Meilan, R., Ellis, D., Pilate, G., Brunner, A., & Skinner, J.S. (2004a)
Accomplishments and challenges in genetic engineering of forest trees. *In* (eds S.H. Strauss & H.D. Bradshaw), pp. 36–41. Resources for the Future Press, Washington DC.

Meilan, R., Han, K.H., Ma, C., DiFazio, S.P., Eaton, J.A., Hoien, E.A., Stanton, B.J., Crockett, R.P., Taylor, M.L., James, R.R., Skinner, J.S., Jouanin, L., Pilate, G., & Strauss, S.H. (2002b)
The CP4 transgene provides high levels of tolerance to Roundup((R)) herbicide in field-grown hybrid poplars. Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere, 32, 6, pp 967-976
<Go to ISI>://000176313300005

Meilan, R., Sabatti, M., Ma, C.P., & Kuzminsky, E. (2004b)
An early-flowering genotype of Populus. Journal of Plant Biology, 47, 1, pp 52-56
<Go to ISI>://000220701600009

Mikaelsen, K. & Aastveit, K. (1957)
Effects of Neutrons and Chronic Gamma-Radiation on Growth and Fertility in Oats and Barley. Hereditas, 43, 2, pp 371-380
<Go to ISI>://A1957XF29300011

Miller, R. (1975)
Systematic anatomy of the xylem and comments on the relationships of Flacourtiaceae. J. Arnold Arbor., 56, pp 20-102

Misko, A.L. & Germida, J.J. (2002)
Taxonomic and functional diversity of pseudomonads isolated from the roots of field-grown canola. Fems Microbiology Ecology, 42, 3, pp 399-407
<Go to ISI>://000179545100008

Mitton, J.B. & Grant, M.C. (1996)
Genetic variation and the natural history of quaking aspen. Bioscience, 46, 1, pp 25-31
<Go to ISI>://A1996TL76600014

Molnar-Lang, M., Barnabas, B., & Rajki, E. (1980)
Changes in the shape shape, volume, weight and the tissue structure of the pistil in the flowers of male-sterile wheats during flowering. Cereal Res. Commun., 8, pp 371–379

Moreau, C., Aksenov, N., Lorenzo, M.G., Segerman, B., Funk, C., Nilsson, P., Jansson, S., & Tuominen, H. (2005)
A genomic approach to investigate developmental cell death in woody tissues of Populus trees. Genome Biology, 6, 4, pp
<Go to ISI>://000228436000011

Muller, C. & Tessier du Cros, E. (1982)
Storage of Populus nigra seed for five years. Ann. Sci. For., 39, pp 179-185

Mullin, T.J. & Bertrand, S. (1998)

Environmental release of transgenic trees in Canada - potential benefits and assessment of biosafety. Forestry Chronicle, 74, 2, pp 203-219
<Go to ISI>://000073508400035

Muravenko, O.V., Lemesh, V.A., Samatadze, T.E., Amosova, A.V., Grushetskaya, Z.E., Popov, K.V., Semenova, O.Y., Khotyuleva, L.V., & Zelenin, A.V. (2003)
Genome comparisons with chromosomal and molecular markers for three closely related flax species and their hybrids. Russian Journal of Genetics, 39, 4, pp 414-421
<Go to ISI>://000182274700008 AND http://www.botanischergarten.ch/EPOBIO-Linum/Muravenko-Comparison-2003.pdf

Murray, B.E. (1980)
Analyses of Meiotic Metaphase in Haploids and Hybrids of Haploid X Diploid Flax (Linum-Usitatissimum). Canadian Journal of Genetics and Cytology, 22, 4, pp 597-606
<Go to ISI>://A1980LG52300010

Murray, B.G., Morrison, I.N., & Brulebabel, A.L. (1995)
Inheritance of Acetyl-Coa Carboxylase Inhibitor Resistance in Wild Oat (Avena-Fatua). Weed Science, 43, 2, pp 233-238
<Go to ISI>://A1995RB70200013

Murray, B.G., Morrison, I.N., & Friesen, L.F. (2002)
Pollen-mediated gene flow in wild oat. Weed Science, 50, 3, pp 321-325
<Go to ISI>://000176184300007 AND http://www.botanischergarten.ch/EPOBIO-Avena/Murray-Geneflow-2002.pdf

Mustakas, G.C., Kirk, L.D., & Booth, A.N. (1968a)
Crambe Seed Processing - Removal of Toxicity by Soda Ash Treatment and Water Extraction. Journal of the American Oil Chemists Society, 45, 8, pp A472-&
<Go to ISI>://A1968B666300078

Mustakas, G.C., Kirk, L.D., & Griffin, E.L. (1968b)
Crambe Seed Processing Improved Feed Meal by Soda Ash Treatment. Journal of the American Oil Chemists Society, 45, 1, pp 53-&
<Go to ISI>://A1968A548100016

Mustakas, G.C., Kirk, L.D., Griffin, E.L., & Booth, A.N. (1976)
Crambe Seed Processing - Removal of Glucosinolates by Water Extraction. Journal of the American Oil Chemists Society, 53, 1, pp 12-16
<Go to ISI>://A1976BE86500003

Mustakas, G.C., Kopas, G., & Robinson, N. (1964)
Prepress-Solvent Extraction of Crambe - First Commercial Trial Run of New Oilseed. Journal of the American Oil Chemists Society, 41, 8, pp 20-&
<Go to ISI>://A19643518B00074

Mustakas, G.C., Kopas, G., & Robinson, N. (1965)
Prepress-Solvent Extraction of Crambe - First Commercial Trial Run of New Oilseed. Journal of the American Oil Chemists Society, 42, 10, pp A550-&
<Go to ISI>://A19656911000001

Mutoh, N., Kimura, M., Oshima, Y., & Iwaki, H. (1985)
Species-Diversity and Primary Productivity in Miscanthus-Sinensis Grasslands .1. Diversity in Relation to Stand Structure and Dominance. Botanical Magazine-Tokyo, 98, 1050, pp 159-170
<Go to ISI>://A1985ALA8700006

Nandi, O.I., Chase, M.W., & Endress, P.K. (1998)
A combined cladistic analysis of angiosperms using rbcL and non-molecular data sets. Annals of the Missouri Botanical Garden, 85, 1, pp 137-212
<Go to ISI>://000074486100014 AND http://www.botanischergarten.ch/EPOBIO/Nandi-Cladistic-Angiospermae-1998.pdf

Nei, M. (1978)
Estimation of Average Heterozygosity and Genetic Distance from a Small Number of Individuals. Genetics, 89, 3, pp 583-590
<Go to ISI>://A1978FK00600010 AND http://www.botanischergarten.ch/EPOBIO-Beta/Nei-Heterozygosity-1978.pdf

Nevo, E. (1998)
Genetic diversity in wild cereals: Regional and local studies and their bearing on conservation ex situ and in situ. Genetic Resources and Crop Evolution, 45, 4, pp 355-370
<Go to ISI>://000075583700010

Nicole, M.C., Hamel, L.P., Morency, M.J., Beaudoin, N., Ellis, B.E., & Seguin, A. (2006)
MAP-ping genomic organization and organ-specific expression profiles of poplar MAP kinases and MAP kinase kinases. Bmc Genomics, 7, pp

<Go to ISI>://000240732300001

Nielsen, K.M., Bones, A.M., Smalla, K., & van Elsas, J.D. (1998)
Horizontal gene transfer from transgenic plants to terrestrial bacteria - a rare event? Fems Microbiology Reviews, 22, 2, pp 79-103
<Go to ISI>://000075605800002 AND http://www.botanischergarten.ch/HorizontalGT/Nielsen-HGT-rare-1998.pdf

Nielsen, K.M., Gebhard, F., Smalla, K., Bones, A.M., & vanElsas, J.D. (1997)
Evaluation of possible horizontal gene transfer from transgenic plants to the soil bacterium Acinetobacter calcoaceticus BD413. Theoretical and Applied Genetics, 95, 5-6, pp 815-821
<Go to ISI>://A1997YF71500013 AND http://www.botanischergarten.ch/HorizontalGT/Nilson-Evaluation-HGT-1997.pdf

Nielsen, K.M., Smalla, K., & van Elsas, J.D. (2000a)
Natural transformation of Acinetobacter sp strain BD413 with cell lysates of Acinetobacter sp., Pseudomonas fluorescens, and Burkholderia cepacia in soil microcosms. Applied and Environmental Microbiology, 66, 1, pp 206-212
<Go to ISI>://000084585800031

Nielsen, K.M., van Elsas, J.D., & Smalla, K. (2000b)
Transformation of Acinetobacter sp strain BD413(pFG4 Delta nptII) with transgenic plant DNA in soil microcosms and effects of kanamycin on selection of transformants. Applied and Environmental Microbiology, 66, 3, pp 1237-1242
<Go to ISI>://000085604800057

Nielsen, K.M., Van Elsas, J.D., & Smalla, K. (2001)
Dynamics, horizontal transfer and selection of novel DNA in bacterial populations in the phytosphere of transgenic plants. Annals of Microbiology, 51, 1, pp 79-94
<Go to ISI>://000169625900007 AND http://www.botanischergarten.ch/HorizontalGT/Nielsen-Dynamics-HGT-2001.pdf

Nishiguchi, M., Yoshida, K., Sumizono, T., & Tazaki, K. (2002)
A receptor-like protein kinase with a lectin-like domain from lombardy poplar: gene expression in response to wounding and characterization of phosphorylation activity. Molecular Genetics and Genomics, 267, 4, pp 506-514
<Go to ISI>://000176944000009

Nonaka, S., Toriyama, K., Tsunewaki, K., & Shimada, T. (1993)
Breeding of Male-Sterile Lines and Their Maintainer Lines by Backcross Method for Hybrid Wheat Production Using an S(V)-Type Cytoplasm and a 1bl-1rs Chromosome. Japanese Journal of Breeding, 43, 4, pp 567-574
<Go to ISI>://A1993MQ70200010

Ockendon, D.J. (1968)
Linum Perenne Ssp Anglicum (Miller) Ockendon - (L Anglicum Miller). Journal of Ecology, 56, 3, pp 871-&
<Go to ISI>://A1968C913800016 AND http://www.botanischergarten.ch/EPOBIO-Linum/Ockendon-Linumperenne-1968.pdf

Ockendon, D.J. (1971)
Cytology and Pollen Morphology of Natural and Artificial Tetraploids in Linum-Perenne Group. New Phytologist, 70, 3, pp 599-&
<Go to ISI>://A1971J424700016

Odenbach, W. (1985)
Weizen - Zuchtziele, Hybridzüchtung, Genreserven, Abstammungslinien in der deutschen Weizenzüchtung. *In Lehrbuch der Züchtung landwirtschaftlicher Kulturpflanzen* (eds W. Hoffman, A. Mudra & W. Plarre), Vol. 2, pp. 51-67. Paul Parey Verlag, Berlin

OECD (1993) Traditional crop breeding practices: an historical review to serve as a baseline for assessing the role of modern biotechnology OECD, Paris, pp

OECD (1999)
Electronic Source: Consensus Document on the Biology of Triticum aestivum (Bread Wheat) (ed E.D. OECD), JOINT MEETING OF THE CHEMICALS COMMITTEE AND THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY
published by: OECD

OECD (2001a)
Electronic Source: CONSENSUS DOCUMENT ON THE BIOLOGY OF BETA VULGARIS L. (SUGAR BEET) (ed E.D. OECD), JOINT MEETING OF THE CHEMICALS COMMITTEE AND THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY
published by: OECD
http://www.olis.oecd.org/olis/2001doc.nsf/43bb6130e5e86e5fc12569fa005d004c/13658e0b81d09680c1256b19003d4eee/$FILE/JT00118011.PDF

OECD (2001b)
Electronic Source: Populus, Consensus Document (ed OECD), Series on Harmonization of Regulatory Oversight in Biotechnology No. 16

published by: OECD
http://www.olis.oecd.org/olis/2000doc.nsf/4f7adc214b91a685c12569fa005d0ee7/c125692700623b74c1256a0600551816/$FILE/JT00103743.PDF AND http://www.botanischergarten.ch/EPOBIO/OECD-Populus-Consensus-2001.pdf AND (http://www.oecd.org/ehs/)

Okubo, A. & Levin, S.A. (1989)
A Theoretical Framework for Data-Analysis of Wind Dispersal of Seeds and Pollen. Ecology, 70, 2, pp 329-338
<Go to ISI>://A1989T900300004

Oostdam, A. & vanderPlas, L.H.W. (1996)
A cell suspension of Linum flavum (L) in phosphate limited continuous culture. Plant Cell Reports, 16, 3-4, pp 188-191
<Go to ISI>://A1996WY17300013

Orshinsky, B.R. & Sadasivaiah, R.S. (1997)
Effect of plant growth conditions, plating density, and genotype on the anther culture response of soft white spring wheat hybrids. Plant Cell Reports, 16, 11, pp 758-762
<Go to ISI>://A1997XW98500005

Owen, F.V. (1945)
Cytoplasmically inherited male sterility in sugar beets. Journal of Agricultural Research, 71, pp 423-440
http://www.botanischergarten.ch/EPOBIO-Beta/Owen-CMS-1945.pdf

Ozias-Akins, P. (2006)
Apomixis: Developmental characteristics and genetics. Critical Reviews in Plant Sciences, 25, 2, pp 199-214
<Go to ISI>://000237020400005 AND NEBIS

Page, E.R., Gallagher, R.S., Kemanian, A.R., Zhang, H., & Fuerst, E.P. (2006)
Modeling site-specific wild oat (Avena fatua) emergence across a variable landscape. Weed Science, 54, 5, pp 838-846
<Go to ISI>://000240538100005 AND NEBIS

Palau, J.L., Perez-Landa, G., Melia, J., Segarra, D., & Millan, M.M. (2006)
A study of dispersion in complex terrain under winter conditions using high-resolution mesoscale and Lagrangian particle models. Atmospheric Chemistry and Physics, 6, pp 1105-1134
<Go to ISI>://000236552200001 AND http://www.botanischergarten.ch/Modelling/Palau-Dispersion-2006.pdf

Park, S., Oh, S., & Han, K.H. (2004)
Large-scale computational analysis of poplar ESTs reveals the repertoire and unique features of expressed genes in the poplar genome. Molecular Breeding, 14, 4, pp 429-440
<Go to ISI>://000226417400008

Pauk, J., Manninen, O., Mattila, I., Salo, Y., & Pulli, S. (1991)
Androgenesis in Hexaploid Spring Wheat F2 Populations and Their Parents Using a Multiple-Step Regeneration System. Plant Breeding, 107, 1, pp 18-27
<Go to ISI>://A1991GG78100003

Paul, E.M., Capiau, K., Jacobs, M., & Dunwell, J.M. (1995)
A study of gene dispersal via pollen in Nicotiana tabacum using introduced genetic markers. Journal of Applied Ecology, 32, pp 875 - 882
AND http://www.botanischergarten.ch/EPOBIO-Nicotiana/Paul-Dispersal-1995.pdf

Pawlowski, W.P. & Somers, D.A. (1998)
Transgenic DNA integrated into the oat genome is frequently interspersed by host DNA. Proceedings of the National Academy of Sciences of the United States of America, 95, 21, pp 12106-12110
<Go to ISI>://000076447900009 AND http://www.botanischergarten.ch/EPOBIO-Avena/Pawlowski-transgenic-DNA-1998.pdf

Pawlowski, W.P., Torbert, K.A., Rines, H.W., & Somers, D.A. (1998)
Irregular patterns of transgene silencing in allohexaploid oat. Plant Molecular Biology, 38, 4, pp 597-607
<Go to ISI>://000075847500009 AND http://www.botanischergarten.ch/EPOBIO-Avena/Pawlowski-Irregular-1997.pdf

Pekrun, C., Lane, P.W., & Lutman, P.J.W. (2005)
Modelling seedbank dynamics of volunteer oilseed rape (Brassica napus). Agricultural Systems, 84, 1, pp 1-20
<Go to ISI>://000227753300001

Pekrun, C., Lutman, P.J.W., Buchse, A., Albertini, A., & Claupein, W. (2006)
Reducing potential gene escape in time by appropriate post-harvest tillage - Evidence from field experiments with oilseed rape at 10 sites in Europe. European Journal of Agronomy, 25, 4, pp 289-298
<Go to ISI>://000241887300001 http://www.botanischergarten.ch/EPOBIO-Brassica/Pekrun-tillage-2006.pdf

Perret, S.J., Valentine, J., Leggett, J.M., & Morris, P. (2003)

Integration, expression and inheritance of transgenes in hexaploid oat (Avena sativa L.) Journal of Plant Physiology,, 160, 8, pp 931-943
http://www.ingentaconnect.com/content/urban/271/2003/00000160/00000008/art00011 AND http://dx.doi.org/10.1078/0176-1617-00880 AND http://www.botanischergarten.ch/EPOBIO-Avena/Perret-Inheritance-2003.pdf

Pickett, A.A. & Galwey, N.W. (1997)
A further evaluation of hybrid wheat. Plant Varieties and Seeds, 10, 1, pp 15-32
<Go to ISI>://A1997WX50800003

Pilger, R. (1954)
Das System der Gramineae. Botanische Jahrbücher, 76, pp 281-384

Poehlmann, M. (1959) Breeding of field crops. Henry Holt and Company,, New York, pp

Pohl, F. (1937)
Die Pollenerzeugung der Windblu¨ tler. Beihefte zum Botanischen Centralblatt, 56, pp 365–370

Prina, A. (2000)
A taxonomic revision of Crambe, sect. Leptocrambe (Brassicaceae). Botanical Journal of the Linnean Society, 133, 4, pp 509-524
<Go to ISI>://000088947400006 NOT available in NEBIS AND EBZ

Pritchard, J.K., Stephens, M., & Donnelly, P. (2000)
Inference of population structure using multilocus genotype data. Genetics, 155, 2, pp 945-959
<Go to ISI>://000087475100039 AND http://www.botanischergarten.ch/EPOBIO-Beta/Pritchard-Population-2000.pdf

Rabbani, M.A., Qureshi, A.A., Afzal, M., Anwar, R., & Komatsu, S. (2001)
Characterization of mustard [Brassica juncea (L.) Czern. & Coss.] germplasm by sds-page of total seed proteins. Pakistan Journal of Botany, 33, 2, pp 173-179
<Go to ISI>://000175872100008

Rajora, O.P. & Dancik, B.P. (1995)
Chloroplast DNA Variation in Populus .1. Intraspecific Restriction Fragment Diversity within Populus-Deltoides, P. Nigra and P. Maximowiczii. Theoretical and Applied Genetics, 90, 3-4, pp 317-323
<Go to ISI>://A1995QR66400003

Rajora, O.P. & Zsuffa, L. (1990)
Allozyme Divergence and Evolutionary Relationships among Populus-Deltoides, Populus-Nigra, and Populus-Maximowiczii. Genome, 33, 1, pp 44-49
<Go to ISI>://A1990CX65300008

Ralph, S., Oddy, C., Cooper, D., Yueh, H., Jancsik, S., Kolosova, N., Philippe, R.N., Aeschliman, D., White, R., Huber, D., Ritland, C.E., Benoit, F., Rigby, T., Nantel, A., Butterfield, Y.S.N., Kirkpatrick, R., Chun, E., Liu, J., Palmquist, D., Wynhoven, B., Stott, J., Yang, G., Barber, S., Holt, R.A., Siddiqui, A., Jones, S.J.M., Marra, M.A., Ellis, B.E., Douglas, C.J., Ritland, K., & Bohlmann, J. (2006)
Genomics of hybrid poplar (Populus trichocarpa x deltoides) interacting with forest tent caterpillars (Malacosoma disstria): normalized and full-length cDNA libraries, expressed sequence tags, and a cDNA microarray for the study of insect-induced defences in poplar. Molecular Ecology, 15, 5, pp 1275-1297
<Go to ISI>://000236584900008

Ramulu, K.S., Sharma, V.K., Naumova, T.N., Dijkhuis, P., & Campagne, M.M.V. (1999)
Apomixis for crop improvement. Protoplasma, 208, 1-4, pp 196-205
<Go to ISI>://000084535500023 AND http://www.botanischergarten.ch/Apomixis/Ramulu-Apomixis-Crop-1999.pdf

Ran, Z. & Michaelis, G. (1995)
Mapping of a Chloroplast Rflp Marker Associated with the Cms Cytoplasm of Sugar-Beet (Beta-Vulgaris). Theoretical and Applied Genetics, 91, 6-7, pp 836-840
<Go to ISI>://A1995TH31900003

Raybould, A.F. & Gray, A.J. (1993)
Genetically-Modified Crops and Hybridization with Wild Relatives - a Uk Perspective. Journal of Applied Ecology, 30, 2, pp 199-219

Raynor, G.S., Hayes, J.V., & Ogden, E.C. (1972)
Dispersion and Deposition of Timothy Pollen from Experimental Sources. Agricultural Meteorology, 9, 5-6, pp 347-&
<Go to ISI>://A1972M086300004

Raynor, G.S., Ogden, E.C., & Hayes, J.V. (1971)
Dispersion and Deposition of Pollens as a Function of Source and Particle Size. Bulletin of the American Meteorological Society, 52, 4, pp 309-&
<Go to ISI>://A1971J346400059

ReamonButtner, S.M., Wricke, G., & Frese, L. (1996)
Interspecific relationship and genetic diversity in wild beets in section Corollinae genus beta: Isozyme and RAPD analyses. Genetic Resources and Crop Evolution, 43, 3, pp 261-274
<Go to ISI>://A1996UT84600008 AND http://www.botanischergarten.ch/EPOBIO-Beta/Reamon-Buettner-Interspecific-1996.pdf

Rechinger, K.H. (1992)
Salix Taxonomy in Europe - Problems, Interpretations, Observations. Proceedings of the Royal Society of Edinburgh Section B-Biological Sciences, 98, pp 1-12
<Go to ISI>://A1992HW57500002 AND http://www.botanischergarten.ch/EPOBIO-Wheat/Rechinger-Problems-1992.pdf

Richards, A. (1986) Pland Breeding Systems George, Allan and Unwin, London, pp 403-456

Roberts, J.K. & Pryor, A. (1995)
Isolation of a Flax (Linum-Usitatissimum) Gene Induced During Susceptible Infection by Flax Rust (Melampsora-Lini). Plant Journal, 8, 1, pp 1-8
<Go to ISI>://A1995RJ58000001

Robinson, A.R., Gheneim, R., Kozak, R.A., Ellis, D.D., & Mansfield, S.D. (2005)
The potential of metabolite profiling as a selection tool for genotype discrimination in Populus. Journal of Experimental Botany, 56, 421, pp 2807-2819
<Go to ISI>://000232748400004

Robinson, C. (1999)
Making forest biotechnology a commercial reality - Do we need a tree genome project, or will Arabidopsis point the way? Nature Biotechnology, 17, 1, pp 27-30
<Go to ISI>://000077979500020

Rodean, H.C. (1995)
Turbulent-Diffusion as a Stochastic Lagrangian Process. Environmetrics, 6, 6, pp 659-663
<Go to ISI>://A1995TJ14400012

Rogers, C.M. (1982)
The Systematics of Linum-Sect Linopsis (Linaceae). Plant Systematics and Evolution, 140, 2-3, pp 225-234
<Go to ISI>://A1982NZ79300010

Romeis, J., Meissle, M., & Bigler, F. (2006)
Transgenic crops expressing Bacillus thuringiensis toxins and biological control. Nature Biotechnology, 24, 1, pp 63-71
<Go to ISI>://000234555800025 AND http://www.botanischergarten.ch/Bt/Romeisetal2006-NB.pdf

Rottmann, W.H., Meilan, R., Sheppard, L.A., Brunner, A.M., Skinner, J.S., Ma, C.P., Cheng, S.P., Jouanin, L., Pilate, G., & Strauss, S.H. (2000)
Diverse effects of overexpression of LEAFY and PTLF, a poplar (Populus) homolog of LEAFY/FLORICAULA, in transgenic poplar and Arabidopsis. Plant Journal, 22, 3, pp 235-245
<Go to ISI>://000087258400006 AND http://www.botanischergarten.ch/EPOBIO/Rottmann-Overexpression-2000.pdf

Sadoch, Z., Goc, A., Wierzchoslawski, R., & Dalke, L. (2003)
Cytoplasmic male sterility in hybrids of sterile wild beet (Beta vulgaris ssp maritima) and O-type fertile sugar beet (Beta vulgaris L.): molecular analysis of mitochondrial and nuclear genomes. Molecular Breeding, 11, 2, pp 137-148
<Go to ISI>://000181041000007

Sadras, V.O. & Monzon, J.P. (2006)
Modelled wheat phenology captures rising temperature trends: Shortened time to flowering and maturity in Australia and Argentina. Field Crops Research, 99, 2-3, pp 136-146
<Go to ISI>://000240562600007

Saeglitz, C., Bartsch, D., Eber, S., Gathmann, A., Priesnitz, K.U., & Schuphan, I. (2006)
Monitoring the Cry1Ab susceptibility of European corn borer in Germany. Journal of Economic Entomology, 99, 5, pp 1768-1773
<Go to ISI>://000241240400035 AND NEBIS

Saeglitz, C., Pohl, M., & Bartsch, D. (2000)
Monitoring gene flow from transgenic sugar beet using cytoplasmic male-sterile bait plants. Molecular Ecology, 9, 12, pp 2035-2040
<Go to ISI>://000166112700010 AND http://www.botanischergarten.ch/EPOBIO-Beta/Saeglitz-Geneflow-2000.pdf

Salick, J. & Pfeffer, E. (1999)
The interplay of hybridization and clonal reproduction in the evolution of willows - Experiments with hybrids of S-eriocephala [R] & S-exigua [X] and S-eriocephala & S-petiolaris [P]. Plant Ecology, 141, 1-2, pp 163-178
<Go to ISI>://000081190600019 AND http://www.botanischergarten.ch/EPOBIO-Salix/Salick-Interplay-1999.pdf

Salonen, V. & Lammi, A. (2001)
Effects of root hemiparasitic infection on host performance: Reduced flower size and increased flower asymmetry. Ecoscience, 8, 2, pp 185-190
<Go to ISI>://000169569300006

Salonen, V. & Suhonen, J. (1995)
Effects of Seed Weight on Growth, Reproduction and Competitive Ability of Linum-Usitatissimum Seedlings. Annales Botanici Fennici, 32, 2, pp 101-106
<Go to ISI>://A1995RR87600003

Sampedro, J., Carey, R.E., & Cosgrove, D.J. (2006)
Genome histories clarify evolution of the expansin superfamily: New insights from the poplar genome and pine ESTs. Journal of Plant Research, 119, 1, pp 11-21
<Go to ISI>://000235407000003

Satoh, M., Kubo, T., & Mikami, T. (2006)
The Owen mitochondrial genome in sugar beet (Beta vulgaris L.): possible mechanisms of extensive rearrangements and the origin of the mitotype-unique regions. Theoretical and Applied Genetics, 113, 3, pp 477-484
<Go to ISI>://000239002300010 AND http://www.botanischergarten.ch/EPOBIO-Beta/Sato-Owen-Mitochondrial-2006.pdf

Saumitoulaprade, P., Rouwendal, G.J.A., Cuguen, J., Krens, F.A., & Michaelis, G. (1993)
Different Cms Sources Found in Beta-Vulgaris Ssp Maritima - Mitochondrial Variability in Wild Populations Revealed by a Rapid Screening-Procedure. Theoretical and Applied Genetics, 85, 5, pp 529-535
<Go to ISI>://A1993KH04900005

Schemske, D.W. & Lande, R. (1985)
The Evolution of Self-Fertilization and Inbreeding Depression in Plants .2. Empirical Observations. Evolution, 39, 1, pp 41-52
<Go to ISI>://A1985ACA4300004 AND http://www.botanischergarten.ch/EPOBIO-Avena/Schemske-SelfFert-Inbreeding-Empiric-1985.pdf

Schmidt, T., Jung, C., HeslopHarrison, J.S., & Kleine, M. (1997)
Detection of alien chromatin conferring resistance to the beet cyst nematode (Heterodera schachtii Schm) in cultivated beet (Beta vulgaris L) using in situ hybridization. Chromosome Research, 5, 3, pp 186-193
<Go to ISI>://A1997XL69900006

Schoenenberger, N., Felber, F., Savova-Bianchi, D., & Guadagnuolo, R. (2005)
Introgression of wheat DNA markers from A, B and D genomes in early generation progeny of Aegilops cylindrica Host x Triticum aestivum L. hybrids. Theoretical and Applied Genetics, 111, 7, pp 1338-1346
<Go to ISI>://000233323900013

Schoenenberger, N., Guadagnuolo, R., Savova-Bianchi, D., Kupfer, P., & Felber, F. (2006)
Molecular analysis, cytogenetics and fertility of introgression lines from transgenic wheat to Aegilops cylindrica host. Genetics, 174, 4, pp 2061-2070
<Go to ISI>://000243284500030

Schönenberger, N. (2005)
Genetic and ecological aspects of gene flow from wheat (Triticum aestivum L.) to Aegilops L. species, University of Neuchatel, Neuchatel Thesis, pp 77
http://www.botanischergarten.ch/Triticum/Schoenenberger-Aegilops-thesis-2005.pdf

Schreiner, E. (1974)
Populus L. - Poplar. *In Seeds of woody plants in the United States* (ed C. Schopmeyer), Vol. Agricultural Hanbook No. 450, pp. 645-655. USDA, Washington DC

Schulz, O.E. (1936)
Cruciferae. *In Die natürlichen Pflanzenfamilien* (eds E. A. & K. Prantl), Vol. 17B, pp. 227-658. Verlag von Wilhelm Engelmann, Leipzig

Scott, R.K. & Longden, P.C. (1970)
Pollen Release by Diploid and Tetraploid Sugar-Beet Plants. Annals of Applied Biology, 66, 1, pp 129-&
<Go to ISI>://A1970H228500015 AND NEBIS

Seethara.A (1971)
Changes in Oil Content and Seed Color Associated with a Mutation for Yellow Seed Coat Color in Linum-Usitatissimum L. Zeitschrift Fur Pflanzenzuchtung, 66, 4, pp 331-&
<Go to ISI>://A1971L397000006

Seethara.A (1972)
Interspecific Hybridization in Linum. Euphytica, 21, 3, pp 489-&
<Go to ISI>://A1972O164000011 AND NEBIS

Seethara.A & Srinivas.D (1972)
Cytomorphological Studies in Genus Linum. Cytologia, 37, 4, pp 661-671
<Go to ISI>://A1972P249800017

Séguin, A., Lapointe, G., & PJ., C. (1998)
Transgenic trees. *In Forest products biotechnology* (eds A. Bruce & J. Palfreyman), pp. 287–303. Taylor and Francis, London

Shorter, R., Gibson, P., & Frey, K.J. (1978)
Outcrossing Rates in Oat Species Crosses (Avena-Sativa L X Avena-Sterilis L). Crop Science, 18, 5, pp 877-878
<Go to ISI>://A1978FW50200051 AND http://www.botanischergarten.ch/EPOBIO-Avena/Shorter-Outcrossing-1978.pdf

Sime, K. & Baldwin, I. (2003)
Opportunistic out-crossing in Nicotiana attenuata (Solanaceae), a predominantly self-fertilizing native tobacco. BMC Ecology, 3, 1, pp 6
http://www.biomedcentral.com/1472-6785/3/6 AND http://www.botanischergarten.ch/EPOBIO-Nicotiana/Syme-Opportunistic-2003.pdf

Sitte, P., Ziegler, H., Ehrendorfer, F., & Bresinsky, A. (1991) Lehrbuch der Botanik für Hochschulen, 33. Auflage edn. Gustav Fischer, Stuttgart, pp 514-514

Sjodin, A., Bylesjo, M., Skogstrom, O., Eriksson, D., Nilsson, P., Ryden, P., Jansson, S., & Karlsson, J. (2006)
UPSC-BASE - Populus transcriptomics online. Plant Journal, 48, 5, pp 806-817
<Go to ISI>://000242042900013

Skvortsov, A.K. (1968) Willows of the USSR., Moscow, pp

Slageren van, M.W. (1994) Wild wheats: a monograph of Aegilops L. and Amblyopyrum (Jaub & Spach) Eig. (Poaceae) Agricultural University, Wageningen, Netherlands, pp

Smalla, K. & Sobecky, P.A. (2002)
The prevalence and diversity of mobile genetic elements in bacterial communities of different environmental habitats: insights gained from different methodological approaches. Fems Microbiology Ecology, 42, 2, pp 165-175
<Go to ISI>://000179450100002 AND http://www.botanischergarten.ch/HorizontalGT/Smalla-Review-HGT-2002.pdf

Smart, J. (1992)
Ecogeographical differentiation and ecotype formation. *In International Beta genetic resources network IBPGR 3-8* (ed L. Frese), Vol. 3-8. IBPGR, Rome

Smith, C.M. & Campbell, M.M. (2004)
Complete nucleotide sequence of the genomic RNA of Poplar mosaic virus (genus Carlavirus). Archives of Virology, 149, 9, pp 1831-1841
<Go to ISI>://000223910100012

Smith, G.A. (1980)
Sugar Beet. *In Hybridization of Crop Plants* (ed A.S.f.A.-C.S.S.o. America), pp. 601-616

Smith, G.A. & Ruppel, E.G. (1980)
Registration of Fc-607 and Fc-607 Cms Sugarbeet Germplasm. Crop Science, 20, 3, pp 419-419
<Go to ISI>://A1980KB55500056

Smith, M.L., Bruhn, J.N., & Anderson, J.B. (1992)
The Fungus Armillaria-Bulbosa Is among the Largest and Oldest Living Organisms. Nature, 356, 6368, pp 428-431
<Go to ISI>://A1992HL83000061

Sobral, B.W.S., Braga, D.P.V., Lahood, E.S., & Keim, P. (1994)
Phylogenetic Analysis of Chloroplast Restriction Enzyme Site Mutations in the Saccharinae Griseb Subtribe of the Andropogoneae Dumort Tribe. Theoretical and Applied Genetics, 87, 7, pp 843-853
<Go to ISI>://A1994MY86300012 AND NEBIS bestellt

Song, K. & Osborn, T.C. (1992)
Polyphyletic Origins of Brassica-Napus - New Evidence Based on Organelle and Nuclear Rflp Analyses. Genome, 35, 6, pp 992-1001
<Go to ISI>://A1992KD50000014

Song, K., Osborn, T.C., & Williams, P.H. (1990)
Brassica Taxonomy Based on Nuclear Restriction Fragment Length Polymorphisms (Rflps) .3. Genome Relationships in Brassica and Related Genera and the Origin of Brassica-Oleracea and B-Rapa (Syn Campestris). Theoretical and Applied Genetics, 79, 4, pp 497-506
<Go to ISI>://A1990DA61600012 AND http://dx.doi.org/10.1007/BF00226159 AND
http://www.botanischergarten.ch/EPOBIO-Brassica/Song-Brassica-Taxonomy-1990.pdf

Song, K.M., Osborn, T.C., & Williams, P.H. (1988)

Brassica Taxonomy Based on Nuclear Restriction Fragment Length Polymorphisms (Rflps) .1. Genome Evolution of Diploid and Amphidiploid Species. Theoretical and Applied Genetics, 75, 5, pp 784-794
<Go to ISI>://A1988N341900022

Soreng, R.J. (1990)
Chloroplast-DNA Phylogenetics and Biogeography in a Reticulating Group - Study in Poa (Poaceae). American Journal of Botany, 77, 11, pp 1383-1400
<Go to ISI>://A1990EJ57900001

Soreng, R.J., Davis, J.I., & Doyle, J.J. (1990)
A Phylogenetic Analysis of Chloroplast DNA Restriction Site Variation in Poaceae Subfam Pooideae. Plant Systematics and Evolution, 172, 1-4, pp 83-97
<Go to ISI>://A1990DY58600008

Spillane, C., Curtis, M.D., & Grossniklaus, U. (2004)
Apomixis technology development - virgin births in farmers' fields? Nature Biotechnology, 22, 6, pp 687-691
<Go to ISI>://000221785300028 AND http://www.botanischergarten.ch/Apomixis/Spillane-Apomixis-Virgin-2004.pdf

Srinivas.D, Seethara.A, & Malik, R.S. (1972)
Combination of 3 Characters (High Oil Content, High Iodine Value, and High-Yield) in a Single Variety of Linseed Linum-Usitatissimum L Obtained by Mutation Breeding. Current Science, 41, 5, pp 169-&
<Go to ISI>://A1972L799200002

Stebbins, L. (1950) Variation and evolution in plants Columbia University Press, New York, pp

Stegnii, V.N., Chudinova, Y.V., & Salina, E.A. (2000)
RAPD analysis of flax (Linum usitatissimum L.) varieties and hybrids of various productivity. Russian Journal of Genetics, 36, 10, pp 1149-1152
<Go to ISI>://000165122700008

Sterck, L., Rombauts, S., Jansson, S., Sterky, F., Rouze, P., & Van de Peer, Y. (2005)
EST data suggest that poplar is an ancient polyploid. New Phytologist, 167, 1, pp 165-170
<Go to ISI>://000229581600017

Sterky, F., Bhalerao, R.R., Unneberg, P., Segerman, B., Nilsson, P., Brunner, A.M., Charbonnel-Campaa, L., Lindvall, J.J., Tandre, K., Strauss, S.H., Sundberg, B., Gustafsson, P., Uhlen, M., Bhalerao, R.P., Nilsson, O., Sandberg, G., Karlsson, J., Lundeberg, J., & Jansson, S. (2004)
A Populus EST resource for plant functional genomics. Proceedings of the National Academy of Sciences of the United States of America, 101, 38, pp 13951-13956
<Go to ISI>://000224069800046 AND http://www.botanischergarten.ch/EPOBIO/Sterky-EST-Genomics-2004.pdf

Stokstad, E. (2006)
Genomics - Poplar tree sequence yields genome double take. Science, 313, 5793, pp 1556-1556
<Go to ISI>://000240498900008

Strauss, S.H., Brunner, A.M., Busov, V.B., Ma, C.P., & Meilan, R. (2004)
Ten lessons from 15 years of transgenic Populus research. Forestry, 77, 5, pp 455-465
<Go to ISI>://000225641600008 AND http://www.botanischergarten.ch/EPOBIO/StraussETAL-TenLessonsForestry2004.pdf

Strauss, S.H., Coventry, P., Campbell, M.M., Pryor, S.N., & Burley, J. (2001a)
Certification of genetically modified forest plantations. Int. Forest. Rev., 3, pp 87–104

Strauss, S.H., DiFazio, S.P., & Meilan, R. (2001b)
Genetically modified poplars in context. Forestry Chronicle, 77, 2, pp 271-279
<Go to ISI>://000168936700013

Strauss, S.H. & Martin, F.M. (2004)
Poplar genomics comes of age. New Phytologist, 164, 1, pp 1-4
<Go to ISI>://000223662000001 AND http://www.botanischergarten.ch/EPOBIO/Strauss-Poplar-Gemonics-Of-Age-2004.pdf

Strauss, S.H., Rottmann, W.H., Brunner, A.M., & Sheppard, L.A. (1995)
Genetic-Engineering of Reproductive Sterility in Forest Trees. Molecular Breeding, 1, 1, pp 5-26
<Go to ISI>://A1995RJ51700003 AND http://www.botanischergarten.ch/EPOBIO/Strauss-Sterility-1995.pdf

Street, N.R., Skogstrom, O., Tucker, J., Rodriguez-Acosta, M., Nilsson, P., Jansson, S., & Taylor, G. (2006)
The genetics and genomics of the drought response in Populus. Plant Journal, 48, 3, pp 321-341
<Go to ISI>://000241240300001

Stringam, G.R. & Downey, R.K. (1978a)

Effectiveness of Isolation Distance in Turnip Rape. Canadian Journal of Plant Science, 58, 2, pp 427-434
<Go to ISI>://A1978FA30300018

Stringam, G.R. & Downey, R.K. (1978b)
Effects of Isolation Distances and Pedigree Seed Production in Rapeseed. Canadian Journal of Plant Science, 58, 2, pp 585-585
<Go to ISI>://A1978FA30300064

Subedi, K.D., Gregory, P.J., Summerfield, R.J., & Gooding, M.J. (1998)
Cold temperatures and boron deficiency caused grain set failure in spring wheat (Triticum aestivum L). Field Crops Research, 57, 3, pp 277-288
<Go to ISI>://000074811900004

Suh, S.W. (2006)
A hybrid approach to particle tracking and Eulerian-Lagrangian models in the simulation of coastal dispersion. Environmental Modelling & Software, 21, 2, pp 234-242
<Go to ISI>://000235131700010 AND http://www.botanischergarten.ch/Modelling/Sue-Hybrid-Models-2004.pdf

Sundback, K., Miles, A., Hulth, S., Pihl, L., Engstrom, P., Selander, E., & Svenson, A. (2003)
Importance of benthic nutrient regeneration during initiation of macroalgal blooms in shallow bays. Marine Ecology-Progress Series, 246, pp 115-126
<Go to ISI>://000181085100009

Suneson, C.A. & Cox, E.L. (1964)
Promiscuity in barley and wheat. Crop Science, 4, pp 233-234

Svitashev, S., Ananiev, E., Pawlowski, W.P., & Somers, D.A. (2000)
Association of transgene integration sites with chromosome rearrangements in hexaploid oat. Theoretical and Applied Genetics, 100, 6, pp 872-880
<Go to ISI>://000087061100007 AND http://www.botanischergarten.ch/EPOBIO-Avena/Svitashev-Rearrangements-2000.pdf

Svitashev, S.K., Pawlowski, W.P., Makarevitch, I., Plank, D.W., & Somers, D.A. (2002)
Complex transgene locus structures implicate multiple mechanisms for plant transgene rearrangement. Plant Journal, 32, 4, pp 433-445
<Go to ISI>://000179414100002

Sylven, N. (1925)
Some splitting numbers with the hybridisation of some blue and white blooming varieties of linum usitatissimum. Hereditas, 7, pp 75-101
<Go to ISI>://000201148600002

Tanksley, S.D., Young, N.D., Paterson, A.H., & Bonierbale, M.W. (1989)
Rflp Mapping in Plant-Breeding - New Tools for an Old Science. Bio-Technology, 7, 3, pp 257-264
<Go to ISI>://A1989T447200015

Tao, Q.Z. & Zhang, H.B. (1998)
Cloning and stable maintenance of DNA fragments over 300 kb in Escherichia coli with conventional plasmid-based vectors. Nucleic Acids Research, 26, 21, pp 4901-4909
<Go to ISI>://000076845300016 AND http://www.botanischergarten.ch/EPOBIO-Beta/Tao-Cloning-1998.pdf

Tauer, C.G. (1979)
Seed tree, vacuum, and temperature effects on eastern cottonwood seed viability during extended storage. Forest Science, 25, pp 112-114

Teeri, T.T. & Brumer, H. (2003)
Discovery, characterisation and applications of enzymes from the wood-forming tissues of poplar: Glycosyl transferases and xyloglucan endotransglycosylases. Biocatalysis and Biotransformation, 21, 4-5, pp 173-179
<Go to ISI>://000187032500005

Thurmann, R.L. & Womak, D. (1961)
Percentage natural crossing in oats produced from irradiated seeds. Crop Science, 1, pp 374

Tingey, S.V. & Deltufo, J.P. (1993)
Genetic-Analysis with Random Amplified Polymorphic DNA Markers. Plant Physiology, 101, 2, pp 349-352
<Go to ISI>://A1993KM09400003

Triest, L., De Greef, B., De Bondt, R., & Van Slycken, J. (2000)
RAPD of controlled crosses and clones from the field suggests that hybrids are rare in the Salix alba-Salix fragilis complex. Heredity, 84, 5, pp 555-563
<Go to ISI>://000087500000007

Tsai, C.J. & Hubscher, S.L. (2004)
Cryopreservation in Populus functional genomics. New Phytologist, 164, 1, pp 73-81
<Go to ISI>://000223662000009

Tschaplinski, T.J., Tuskan, G.A., Sewell, M.M., Gebre, G.M., Donald, E.T.I., & Pendleyi, C. (2006)
Phenotypic variation and quantitative trait locus identification for osmotic potential in an interspecific hybrid inbred F-2 poplar pedigree grown in contrasting environments. Tree Physiology, 26, 5, pp 595-604
<Go to ISI>://000237369400006

Tsunewaki, K. (1969)
Basic studies on hybrid wheat breeding. IV. Natural cross-fertilization in male-sterile wheat. (In Japanese). Seiken Jiho, 21, pp 1-5

Tsunewaki, K. (1993)
Genome-Plasmon Interactions in Wheat. Japanese Journal of Genetics, 68, 1, pp 1-34
<Go to ISI>://A1993LE58400001

Tsvelev, N.N. (1989)
The system of grasses (Poaceae) and their evolution. Botanical Review, 55, pp 141-204

Tuskan, G.A., DiFazio, S., Jansson, S., Bohlmann, J., Grigoriev, I., Hellsten, U., Putnam, N., Ralph, S., Rombauts, S., Salamov, A., Schein, J., Sterck, L., Aerts, A., Bhalerao, R.R., Bhalerao, R.P., Blaudez, D., Boerjan, W., Brun, A., Brunner, A., Busov, V., Campbell, M., Carlson, J., Chalot, M., Chapman, J., Chen, G.L., Cooper, D., Coutinho, P.M., Couturier, J., Covert, S., Cronk, Q., Cunningham, R., Davis, J., Degroeve, S., Dejardin, A., Depamphilis, C., Detter, J., Dirks, B., Dubchak, I., Duplessis, S., Ehlting, J., Ellis, B., Gendler, K., Goodstein, D., Gribskov, M., Grimwood, J., Groover, A., Gunter, L., Hamberger, B., Heinze, B., Helariutta, Y., Henrissat, B., Holligan, D., Holt, R., Huang, W., Islam-Faridi, N., Jones, S., Jones-Rhoades, M., Jorgensen, R., Joshi, C., Kangasjarvi, J., Karlsson, J., Kelleher, C., Kirkpatrick, R., Kirst, M., Kohler, A., Kalluri, U., Larimer, F., Leebens-Mack, J., Leple, J.C., Locascio, P., Lou, Y., Lucas, S., Martin, F., Montanini, B., Napoli, C., Nelson, D.R., Nelson, C., Nieminen, K., Nilsson, O., Pereda, V., Peter, G., Philippe, R., Pilate, G., Poliakov, A., Razumovskaya, J., Richardson, P., Rinaldi, C., Ritland, K., Rouze, P., Ryaboy, D., Schmutz, J., Schrader, J., Segerman, B., Shin, H., Siddiqui, A., Sterky, F., Terry, A., Tsai, C.J., Uberbacher, E., Unneberg, P., et al. (2006)
The genome of black cottonwood, Populus trichocarpa (Torr. & Gray). Science, 313, 5793, pp 1596-1604
<Go to ISI>://000240498900035

Tuskan, G.A., DiFazio, S.P., & Teichmann, T. (2004)
Poplar genomics is getting popular: The impact of the poplar genome project on tree research. Plant Biology, 6, 1, pp 2-4
<Go to ISI>://000220061800002

Tzfira, T., Zuker, A., & Atman, A. (1998)
Forest-tree biotechnology: genetic transformation and its application to future forests. Trends in Biotechnology, 16, 10, pp 439-446
<Go to ISI>://000076491000008 AND http://www.botanischergarten.ch/EPOBIO/Tzfira-Treebiotech-1998.pdf

U, N. (1935)
Genome analysis in Brassica with special reference to the experimental formation of B. napus and peculiar mode of fertilization, in Japanese. Journal of Botany, 7, pp 389-452

Valdeyron, G. (1884)
Production de semences. *In Pollinisation et productions végétales* (eds P. Pesson & J. Louveaux), pp. 143-162. INRA, Paris

van Dijk, P. & van Damme, J. (2000)
Apomixis technology and the paradox of sex. Trends in Plant Science, 5, 2, pp 81-84
<Go to ISI>://000085254000008 AND http://www.botanischergarten.ch/Apomixis/vanDijk-Apomixis-2000.pdf

Vanden Broeck, A., Storme, V., Cottrell, J.E., Boerjan, W., Van Bockstaele, E., Quataert, P., & Van Slycken, J. (2004)
Gene flow between cultivated poplars and native black poplar (Populus nigra L.): a case study along the river Meuse on the Dutch-Belgian border. Forest Ecology and Management, 197, 1-3, pp 307-310
<Go to ISI>://000223382700027 AND http://www.botanischergarten.ch/EPOBIO/VandenBroeck-Geneflow-2004.pdf

Viard, F., Arnaud, J.F., Delescluse, M., & Cuguen, J. (2004)
Tracing back seed and pollen flow within the crop-wild Beta vulgaris complex: genetic distinctiveness vs. hot spots of hybridization over a regional scale. Molecular Ecology, 13, 6, pp 1357-1364
<Go to ISI>://000221302600002 AND http://www.botanischergarten.ch/EPOBIO-Beta/Viard-Tracing-2004.pdf

Viard, F., Bernard, J., & Desplanque, B. (2002)
Crop-weed interactions in the Beta vulgaris complex at a local scale: allelic diversity and gene flow within sugar beet fields. Theoretical and Applied Genetics, 104, 4, pp 688-697
<Go to ISI>://000174791900025 AND http://www.botanischergarten.ch/EPOBIO-Beta/Viard-Interactions-2002.pdf

Villar, M., Gaget, M., & Dumas, C. (1989)

Sexual Reproduction in Populus-I Some Physiological and Biochemical Events of the Progamic Phase. Annales Des Sciences Forestieres, 46, pp S64-S66
<Go to ISI>://A1989CU99900014 AND http://www.botanischergarten.ch/EPOBIO/Villar-Reproduction-1989.pdf

Virmani, S.S. & Edwards, I.B. (1983)
Current Status and Future-Prospects for Breeding Hybrid Rice and Wheat. Advances in Agronomy, 36, pp 145-214
<Go to ISI>://A1983SA25200004

Vollmann, J. & Ruckenbauer, P. (1991)
Estimation of Outcrossing Rates in Crambe (Crambe-Abyssinica Hochst Ex Re Fries) Using a Dominant Morphological Marker Gene. Bodenkultur, 42, 4, pp 361-366
<Go to ISI>://A1991GU34400007 AND http://www.botanischergarten.ch/EPOBIO-Crambe/Vollmann-Outcrossing-1981.pdf

Vonbothmer, R., Gustafsson, M., & Snogerup, S. (1995)
Brassica Sect Brassica (Brassicaceae) .2. Interspecific and Intraspecific Crosses with Cultivars of Brassica-Oleracea. Genetic Resources and Crop Evolution, 42, 2, pp 165-178
<Go to ISI>://A1995RY06900009

Wagner, H., Gimbel, E.M., & Wricke, G. (1989)
Are Beta Procumbens Chr Sm and Beta-Webbiana Moq Different Species. Plant Breeding, 102, 1, pp 17-21
<Go to ISI>://A1989T350300003 AND http://www.botanischergarten.ch/EPOBIO-Beta/Wagner-procumbens-1989.pdf

Waines, J.G. & Hegde, S.G. (2003)
Intraspecific gene flow in bread wheat as affected by reproductive biology and pollination ecology of wheat flowers. Crop Science, 43, 2, pp 451-463
<Go to ISI>://000181479700001 AND http://www.botanischergarten.ch/EPOBIO-Wheat/Waines-Intraspecific-2003.pdf

Wang, G.J., Castiglione, S., Chen, Y., Li, L., Han, Y.F., Tian, Y.C., Gabriel, D.W., Han, Y.N., Mang, K.Q., & Sala, F. (1996)
Poplar (Populus nigra L) plants transformed with a Bacillus thuringiensis toxin gene: Insecticidal activity and genomic analysis. Transgenic Research, 5, 5, pp 289-301
<Go to ISI>://A1996VK44200002 AND NEBIS

Wang, Y.P., Tang, J.S., Chu, C.Q., & Tian, J. (2000)
A preliminary study on the introduction and cultivation of Crambe abyssinica in China, an oil plant for industrial uses. Industrial Crops and Products, 12, 1, pp 47-52
<Go to ISI>://000087544800007 AND http://www.botanischergarten.ch/EPOBIO-Crambe/Wang-China-2000.pdf

Warwick, S.I. & Al-Shehbaz, I.A. (2006)
Brassicaceae: Chromosome number index and database on CD-Rom. Plant Systematics and Evolution, 259, 2-4, pp 237-248
<Go to ISI>://000238998800009 AND http://www.botanischergarten.ch/EPOBIO-Brassica/Warwick-Chromosome-Checklist-2006.pdf

Warwick, S.I. & Black, L.D. (1991)
Molecular Systematics of Brassica and Allied Genera (Subtribe Brassicinae, Brassiceae) - Chloroplast Genome and Cytodeme Congruence. Theoretical and Applied Genetics, 82, 1, pp 81-92
<Go to ISI>://A1991FT42100014 AND http://www.botanischergarten.ch/EPOBIO-Brassica/Warwick-Chloroplast-1991.pdf

Warwick, S.I. & Black, L.D. (1997a)
Molecular phylogeny of tribe Brassiceae (Brassicaceae); tribal status of Calepina, Conringia and Orychophragmus. American Journal of Botany, 84 Suppl., pp 243-244

Warwick, S.I. & Black, L.D. (1997b)
Phylogenetic implications of chloroplast DNA restriction site variation in subtribes Raphaninae and Cakilinae (Brassicaceae, tribe Brassiceae). Canadian Journal of Botany-Revue Canadienne De Botanique, 75, 6, pp 960-973
<Go to ISI>://A1997XP66700012

Warwick, S.I., Black, L.D., & Aguinagalde, I. (1992)
Molecular Systematics of Brassica and Allied Genera (Subtribe Brassicinae, Brassiceae) - Chloroplast DNA Variation in the Genus Diplotaxis. Theoretical and Applied Genetics, 83, 6-7, pp 839-850
<Go to ISI>://A1992HP30700025 AND NEBIS

Warwick, S.I., Francis, A., & Al-Shehbaz, I.A. (2006)
Brassicaceae: Species checklist and database on CD-Rom. Plant Systematics and Evolution, 259, 2-4, pp 249-258
<Go to ISI>://000238998800010 AND http://www.botanischergarten.ch/EPOBIO-Brassica/Warwick-Species-Checklist-2006.pdf

Web of Science (2006)
Electronic Source: Bibliography Web of Science: POPULUS and Poplar,
http://www.botanischergarten.ch/EPOBIO/Bibliography-WOS-POPULUS-POPLAR.pdf

Webb, A. (1964)
Cytology of Shoot Apex of Linum. American Journal of Botany, 51, 6P2, pp 675-&
<Go to ISI>://A19642672A00160

Weber, C.R. & Hanson, W.D. (1961)
Natural hybridization with and wiithout ionizing radiation in soybeans. Crop Science, 1, pp 389-392

Weihe, A., Meixner, M., Wolowczyk, B., Melzer, R., & Borner, T. (1991)
Rapid Hybridization-Based Assays for Identification by DNA Probes of Male-Sterile and Male-Fertile Cytoplasms of the Sugar-Beet Beta-Vulgaris L. Theoretical and Applied Genetics, 81, 6, pp 819-824
<Go to ISI>://A1991FT42000018

Weising, K., Nybom, N., Wolff, K., & Meyer, W. (1995) DNA fingerprinting in plants and fungi CRC Press, Boca Raton, Fla., pp

Welsh, J. & McClelland, M. (1990)
Fingerprinting Genomes Using Pcr with Arbitrary Primers. Nucleic Acids Research, 18, 24, pp 7213-7218
<Go to ISI>://A1990EQ47900001

Westgate, M.E., Passioura, J.B., & Munns, R. (1996)
Water status and ABA content of floral organs in drought-stressed wheat. Australian Journal of Plant Physiology, 23, 6, pp 763-772
<Go to ISI>://A1996VZ37000010

White, G.A. (1975)
Distinguishing Characteristics of Crambe-Abyssinica and Crambe-Hispanica. Crop Science, 15, 1, pp 91-93
<Go to ISI>://A1975V767000028 AND http://www.botanischergarten.ch/EPOBIO-Crambe/White-Distinguishing-1975.pdf

White, G.A. & Solt, M. (1978)
Chromosome-Numbers in Crambe, Crambella, and Hemicrambe. Crop Science, 18, 1, pp 160-161
<Go to ISI>://A1978EV03700044 AND NEBIS

Wilkinson, M., Harding, K., Obrien, E., Dubbels, S., Charters, Y., & Lawson, H. (1993)
Herbicides and Transgenic Rape. Nature, 365, 6442, pp 114-114
<Go to ISI>://A1993LW44200031 AND NEBIS

Wilkinson, M.J., Davenport, I.J., Charters, Y.M., Jones, A.E., Allainguillaume, J., Butler, H.T., Mason, D.C., & Raybould, A.F. (2000)
A direct regional scale estimate of transgene movement from genetically modified oilseed rape to its wild progenitors. Molecular Ecology, 9, 7, pp 983-991
<Go to ISI>://000088579300014 AND http://www.botanischergarten.ch/EPOBIO-Brassica/Wilkinson-Brassica-Regionalscale-2000.pdf

Wilkinson, M.J., Elliott, L.J., Allainguillaume, J., Shaw, M.W., Norris, C., Welters, R., Alexander, M., Sweet, J., & Mason, D.C. (2003)
Hybridization between Brassica napus and B-rapa on a national scale in the United Kingdom. Science, 302, 5644, pp 457-459
<Go to ISI>://000185963200046 AND http://www.botanischergarten.ch/EPOBIO-Brassica/Wilkinson-Hybridization-2003.pdf

Willenborg, C.J. & Van Acker, R.C. (2006)
Comments on "An empirical model for pollen-mediated gene flow in wheat" (Crop Sci. 45 : 1286-1294). Crop Science, 46, 2, pp 1018-1019
<Go to ISI>://000235991100090 AND http://www.botanischergarten.ch/EPOBIO-Wheat/Willienborg-Response-Gustafson.pdfNEBIS

Williams, J.T., Scott, A.J., & Ford-Lloyd, B.V. (1976)
Patellaria: a new genus in the Chenopodiacea. Feddes Repertorium, B 87, pp 289-292
http://www.botanischergarten.ch/EPOBIO-Beta/Williams-Patellaria-1976.pdf

Wilson, J.A. (1968)
Problems in hybrid wheat breeding. Euphytica, 17, pp 13-34

Wood, D. & Lenne, J. (2001)
Nature's fields: a neglected model for increasing food production. Outlook on Agriculture, 30, 3, pp 161-170
<Go to ISI>://000171396200003 AND http://www.botanischergarten.ch/Organic/Wood-Natures-Fields-2001.pdf

Wullschleger, S.D., Jansson, S., & Taylor, G. (2002)
Genomics and forest biology: Populus emerges as the perennial favorite. Plant Cell, 14, 11, pp 2651-2655
<Go to ISI>://000179264700002 AND http://www.botanischergarten.ch/EPOBIO/Wullschleger-Genomics-2002.pdf

Xynias, I.N., Zamani, I.A., Gouli-Vavdinoudi, E., & Roupakias, D.G. (2001)

Effect of cold pretreatment and incubation temperature on bread wheat (Triticum aestivum L.) anther culture. Cereal Research Communications, 29, 3-4, pp 331-338
<Go to ISI>://000172706700013

Yang, D.I., Feng, T.Y., Chen, C.C., & Lai, Y.K. (1992)
Physical Maps of Nicotiana Chloroplast DNA Constructed by an Efficient Procedure. Theoretical and Applied Genetics, 83, 4, pp 515-527
<Go to ISI>://A1992HD65400016

Yang, X.H., Chen, X.Y., Ge, Q.Y., Li, B., Tong, Y.P., Zhang, A.M., Li, Z.S., Kuang, T.Y., & Lu, C.M. (2006)
Tolerance of photosynthesis to photoinhibition, high temperature and drought stress in flag leaves of wheat: A comparison between a hybridization line and its parents grown under field conditions. Plant Science, 171, 3, pp 389-397
<Go to ISI>://000239475500012

Yermanos, D.M. & Gill, K.S. (1969)
Cytology of Autotetraploids of Linum Usitatissimum L and Linum Angustifolium Huds and Their Amphidiploid Hybrids. Crop Science, 9, 2, pp 249-&
<Go to ISI>://A1969D189600044

Yilmaz, O. & Kaynak, G.N. (2006a)
Linum hirsutum subsp platyphyllum, stat. nov. (Linaceae). Annales Botanici Fennici, 43, 1, pp 62-63
<Go to ISI>://000237054100007

Yilmaz, O. & Kaynak, G.N. (2006b)
New combination in Linum sect. Syllinum (Linaceae). Annales Botanici Fennici, 43, 1, pp 77-79
<Go to ISI>://000237054100011

Yin, T.M., DiFazio, S.P., Gunter, L.E., Riemenschneider, D., & Tuskan, G.A. (2004)
Large-scale heterospecific segregation distortion in Populus revealed by a dense genetic map. Theoretical and Applied Genetics, 109, 3, pp 451-463
<Go to ISI>://000223097700001

Yin, T.M., Zhang, X.Y., Huang, M.R., Wang, M.X., Zhuge, Q., Tu, S.M., Zhu, L.H., & Wu, R.L. (2002)
Molecular linkage maps of the Populus genome. Genome, 45, 3, pp 541-555
<Go to ISI>://000175373300012

Yu, Y.L. & Lin, T.Y. (1997)
Construction of phylogenetic tree for Nicotiana species based on RAPD markers. Journal of Plant Research, 110, 1098, pp 187-193
<Go to ISI>://A1997XL39800004 AND http://www.botanischergarten.ch/EPOBIO-Nicotiana/Yu-Phylogenetic-1997.pdf

Zalesny, R.S. & Wiese, A.H. (2006)
Date of shoot collection, genotype, and original shoot position affect early rooting of dormant hardwood cuttings of Populus. Silvae Genetica, 55, 4-5, pp 169-182
<Go to ISI>://000240729800004

Zeller, F.J. & Friebe, B. (1991)
Evolution und Züchtung des Saatweizens (Triticum aestivum L.). Biologie in unserer Zeit, 21, 5, pp 248-254
http://dx.doi.org/10.1002/biuz.19910210509 AND NEBIS

Zeng, C.L., Wang, J.B., Liu, A.H., & Wu, X.M. (2004)
Seed coat microsculpturing changes during seed development in diploid and amphidiploid Brassica species. Annals of Botany, 93, 5, pp 555-566
<Go to ISI>://000221084800008

Zhang, D., Zhang, Z., Yang, K., & Li, B. (2004)
Genetic mapping in (Populus tomentosa x Populus bolleana) and P-tomentosa Carr. using AFLP markers. Theoretical and Applied Genetics, 108, 4, pp 657-662
<Go to ISI>://000189207100011

Zhang, S., Cho, M.J., Koprek, T., Yun, R., Bregitzer, P., & Lemaux, P.G. (1999)
Genetic transformation of commercial cultivars of oat (Avena sativa L.) and barley (Hordeum vulgare L.) using in vitro shoot meristematic cultures derived from germinated seedlings. Plant Cell Reports, 18, 12, pp 959-966
<Go to ISI>://000082847500001 AND http://www.botanischergarten.ch/EPOBIO-Avena/Zhang-Avena-Tranformation-1999.pdf

Zhang, Y., Zhang, S.G., Qi, L.W., Liu, B., Gao, J.M., Chen, C.B., Li, X.L., & Song, W.Q. (2005)
Construction of poplar (Populus tremula) chromosome 1 - Specific DNA library by using a microdissection technique. Plant Molecular Biology Reporter, 23, 2, pp 129-138
<Go to ISI>://000233144500003

Zhao, H., Wu, Z., Wu, W., Xie, X., Ma, M., Godovikova, V.A., Lu, M., & Wang, H. (2000)
Ecological Safety Assessment of herbicide resistant wheat - II studies on interspecific and intergeneric hybridization between common wheat and its wild relatives. Journal of Hebei Agricultural Sciences, 4, pp 6-9

Zhao, H.Y., Lu, J., Lu, S.Y., Zhou, Y.H., Wei, J.H., Song, Y.R., & Wang, T. (2005a)
Isolation and functional characterization of a cinnamate 4-hydroxylase promoter from Populus tomentosa. Plant Science, 168, 5, pp 1157-1162
<Go to ISI>://000228528700002

Zhao, J.J., Wang, X.W., Deng, B., Lou, P., Wu, J., Sun, R.F., Xu, Z.Y., Vromans, J., Koornneef, M., & Bonnema, G. (2005b)
Genetic relationships within Brassica rapa as inferred from AFLP fingerprints. Theoretical and Applied Genetics, 110, 7, pp 1301-1314
<Go to ISI>://000228846300017

Zhidkova, E.N. (1997)
Theoretical and practical aspects of remote hybridization of rapeseed. Genetika, 33, 1, pp 5-11
<Go to ISI>://A1997WU32200001

Zohary, D. & Hopf, M. (1994) Domestication of plants in the old world. The origin and spread of cultivated plants in West Asia, Europe and the Nile Valley, 2nd Edition edn. Clarendon Press, Oxford, UK, pp

Zsuffa, L. (1975a)
A summary review of interspecific breeding in the genus Populus L., Dept. Environment, Canadian Forestry Service, Ottawa, Proceedings 14th meeting of the Canadian Tree Improvement Association, part 2, Ed. D. Fowler & C. Yeatman pp 107-123

Zsuffa, L. (1975b)
A summary review of interspecific breeding in the genus Populus L., Dept. Environment, Canadian Forestry Service, Ottawa, Proceedings 14th
meeting of the Canadian Tree Improvement Association, part 2, Ed. pp 107-123

Zueghart, W. & Breckling, B. (2003)
Konzeptionelle Entwicklung eines Monitoring von Umweltwirkungen transgener Kulturpflanzen, Teil 1, UMWELTFORSCHUNGSPLAN DES BUNDESMINISTERIUMS FÜR UMWELT, NATURSCHUTZ UND REAKTORSICHERHEIT pp 201 Forschungsbericht 299 89 406 UBA-FB 000500/ Bremen (Report) http://www.umweltdaten.de/publikationen/fpdf-l/2350.pdf AND http://www.botanischergarten.ch/EPOBIO-Beta/Zueghart-Monitoring-2003.pdf

Zuzens, D., Grant, M.N., McBean, D.S., Shebeski, L.H., & Hurd, E.A. (1969)
Wind Pollination of Male Sterile Wheat. Canadian Journal of Plant Science, 49, 1, pp 98-&
<Go to ISI>://A1969C454300016